THE BRITISH VETERINARY JOURNAL

EDITORS:
J. McCUNN, *F.R.C.S., L.R.C.P., M.R.C.V.S.*
R. LOVELL, *D.Sc., Ph.D., M.R.C.V.S., D.V.S.M.*
SIR THOMAS DALLING, *M.A., D.Sc., F.R.C.V.S. F.R.S.E.,*

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EDITORIAL

PRACTITIONERS AND RESEARCH

Most of the patients who are examined by a general practitioner, be the latter a doctor of men or animals, are afflicted by a common or simple disorder or disease which will respond satisfactorily to good nursing and ordinary therapeutic measures. Many of these conditions can be recognised by a person who has not followed a rigorous professional training; many have been given names which have been accepted as adequate for a long time and are now in common use. In a great number of cases the cause of the disorder is not known, neither do we know in truth how the patient recovers.

It is remarkable that in spite of the great advances that have been made in scientific knowledge we know less about many of the common things than we do of those that are rare. This is true in all walks of life but it is particularly so with regard to all branches of the healing art. Collectively, the minor disorders are responsible for most of the inefficiencies and sufferings of men and animals and the economic repercussions are stupendous. Why are these things allowed to extort their unremitting toll of suffering and economic loss?

It may be because the minor and common ailments do not register their effects, from time to time, in great casualty lists which arrest attention but in such a slow and insidious manner that they are accepted as inevitable and of no great consequence.

(The February issue of The British Veterinary Journal was published on February 20)

It may be that they do not offer a field which is sufficiently impelling or attractive to engage the attention of research workers.

One of the best features of modern research is the tendency for the workers to conduct their investigations as a team and for the individual members of the team to specialise in some particular item or theme. Now this method of conducting investigations has its dangers also. When a man becomes a member of a team he has to subordinate his own views and ideas to a great degree so that they will conform to the plan adopted for the scheme as a whole. He must play the part which has been cast for him and control his own dreams and visions. There is a danger that in spite of becoming more astute and proficient in one specialised branch he may become limited in his knowledge of the whole.

For these reasons medical and veterinary men who confine their attention to pure research are apt to lose touch with the ordinary routine work of their professions and one can understand why some of the common things which are the bane of the practitioner's life receive so little attention.

We should not condemn the research workers for the nature of their work often tends to lead them into academic and fundamental fields which appear to be far removed from the routine or day-to-day hazards and irritations of a practitioner's life. They look to the practitioners to indicate those things which require and justify scientific investigation because of their toll of suffering or economic loss. When the task is indicated and the research worker concentrates all his energies and facilities on the problem the solution may be disclosed in a very short space of time. The modern methods for the control and cure of "Foul in the foot" and "Foot rot" are striking examples of this.

Practitioners may also play their part. They may not have the time and facilities to follow any particular long-term programme of research but they can do much by being adventurous clinicians. They can use their powers of observation and judgment, and the therapeutic methods of trial and error. Sometimes they may achieve results for which no scientific reason can be given immediately but which act as a beacon to a research worker who is struggling to elucidate the natural history of the disease or of some similar pathological process. For this reason THE BRITISH VETERINARY JOURNAL welcomes the records of practitioners who have been able to alleviate suffering and/or economic loss even if their methods might be classed as empirical.

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GENERAL ARTICLES

CHANGES ASSOCIATED WITH AGE IN THE BLOOD PICTURE OF CALVES AND HEIFERS

By H. H. HOLMAN

Agricultural Research Council Field Station, Compton, Nr. Newbury, Berks.

Introduction

LITTLE work has been done on the changes in the blood picture occurring at different ages in cattle. In this country Fraser (1930) recorded the blood picture of Shorthorn calves at four different ages and, recently, Greatorex (1954) has recorded weekly averages provided by a very full investigation of 233 samples from calves. Wirth (1950), in his book on comparative hæmotology, only records a few general results which he attributes to Jedlicka and these are referred to where appropriate.

This paper records the changes observed in calves and heifers of the Ayrshire breed over the first two and a half years of life, and compares these results with those obtained by other workers and with the results obtained for the cow as recorded in the previous paper (Holman, 1955).

Animals and Methods

Animals. Samples were obtained from Ayrshire cattle kept at the A.R.C. Field Station. Male animals were not included in the investigation. Small groups of calves or heifers of different ages were sampled at intervals over periods up to one year. The source of the samples for each age, whether from the same or different individuals, cannot easily be shown in a table and the variety of samples is such that calves recorded at two months (for example) represents seven calves recorded in different months of three different years. Groups were continually being broken up by the requirements of farm practice and occasionally animals would be rejected through illness. This method of sampling increased the variation in samples but it ensured that temporary changes were not due to seasonal variation or to a temporary defect in technique.

Methods. The hæmatological technique was identical with that recorded in the paper on cows' blood (Holman, 1955).

Recordings. The figure is based on the averages recorded in Table I, the line subsequent to the second week was smoothed by the use of three-point moving averages. All the constituents were plotted on the same scale logarithmic paper and therefore a ten per cent rise in any constituent is of equal height. Standard deviations, where used, refer to those of the distribution and not the mean.

Results

The main results are recorded in Table I and in the figure.

Erythrocytic Picture.

Changes in Corpuscular Size. The most definite change in the figure is that of the mean corpuscular volume. This index is unaffected by changes in

TABLE I

Averages for each Blood Constituent for Calves and Heifers at different Ages.
*For viscosity and clotting time the number of samples were less than those recorded in the second column.

AGE	NUMBERS OF SAMPLES	ERYTHROCYTIC PICTURE					PHYSICAL				LEUCOCYTIC PICTURE					
		P.C.V. per cent.	R.C. 10^6 per c.mm	M.C.V. c./u	Hb %/100 ml.	M.C.H.C. %	S.G. 1.0500 recorded as 500	FRAGILITY per cent. NaCl	*VISCOSITY Ag.= 1	*CLOTTING Minutes	WHITE COUNT 10^3 per c.mm	DIFFERENTIAL COUNT				
												Neut.	Lym.	Mono	Eos.	Baso
Day of birth	11	42.5	9.82	44.9	12.9	30.6	500	.346	8.7	7.0	12.9	58.3	32.7	3.7	0.2	0.4
1 day	11	40.8	9.51	43.6	11.5	31.5	517	.351	8.9	9.2	11.3	50.2	40.0	3.9	0.5	0.3
2 or 3 days	10	37.8	8.40	45.4	11.4	35.3	531	.360	6.8	7.1	10.4	43.0	39.9	12.6	1.1	0.3
4 days	12	36.0	9.36	39.6	11.3	31.4	516	.359	6.6	9.4	10.0	43.9	43.6	10.0	0.5	0.7
1 week	12	35.5	9.44	39.4	10.4	30.1	511	.369	6.4	7.0	11.4	43.2	47.4	7.4	0.2	0.7
2 weeks	7	37.0	9.96	34.7	11.0	33.9	500	.380	5.8	9.9	9.5	40.5	54.7	3.8	0.3	0.1
3 weeks	6	32.0	9.15	34.3	10.5	32.8	493	.377		10.1	8.7	38.3	54.4	5.5	0.3	0.3
4 weeks	7	29.4	9.00	33.7	10.1	33.7	477	.420	5.0	8.9	8.7	33.0	59.9	5.8	0.2	0.1
6 weeks	5	29.0	9.42	31.2	10.5	36.5	476	.406		14.0	7.1	27.7	59.9	3.7	0.4	0.0
2 months	7	29.1	9.60	30.8	10.3	35.9	464	.437	4.3	11.6	9.0	23.9	68.4	5.9	0.6	0.7
3	4	34.1	9.52	37.2	11.7	33.0	525	.513	5.8	5.4	12.1	18.5	71.5	8.8	0.4	0.3
4	5	37.1	10.62	35.4	11.7	31.2	525	.528	6.2	5.3	12.1	15.5	75.3	7.5	0.3	0.4
5	5	36.0	9.90	39.4	10.6	29.4	525	.532	6.4	5.6	10.5	21.4	68.9	8.8	0.6	0.2
6	5	34.8	8.04	40.8	10.6	31.8	535	.522	5.9	5.9	11.5	19.8	71.1	7.3	0.2	0.2
7	6	33.2	7.91	40.0	10.4	30.8	533	.523	5.0	7.1	11.8	18.7	71.8	8.6	0.6	0.5
8	6	33.6	8.43	40.1	10.8	32.8	500	.508	4.8	5.1	12.2	19.3	71.2	7.6	1.5	0.4
9	6	33.6	8.05	41.8	11.1	32.1	500	.507	6.1	6.3	12.6	21.0	71.3	5.8	1.2	1.3
10	7	35.7	8.14	42.1	10.3	31.3	500	.509	5.7	5.6	13.0	23.8	66.0	9.2	2.2	1.1
11	8	35.7	8.11	43.1	11.4	32.6	514	.503	5.4	6.1	12.4	23.1	66.9	6.8	2.1	0.9
12	7	35.9	7.65	43.5	10.4	31.6	509	.508	5.7	7.7	11.5	23.3	66.8	5.9	3.4	0.8
13	6	33.5	7.64	44.6	10.6	31.0	507	.487	6.2	6.3	11.5	23.6	62.9	6.8	4.7	1.3
14	6	34.0	7.18	44.5	10.5	32.7	479	.487	5.4	6.1	9.9	24.3	64.7	6.0	3.3	0.7
15	6	33.5	7.75	45.8	10.7	30.2	509	.490	6.2	6.6	10.7	19.7	69.4	5.4	4.7	1.0
16	6	34.1	7.03	49.0	10.3	30.5	500	.482	5.5	6.4	9.9	25.1	65.5	6.2	3.4	0.2
17	7	33.9	7.03	48.9	11.4	34.7	509	.483	5.9	6.8	9.5	24.6	62.5	5.4	6.0	1.0
18	6	33.1	7.20	49.3	10.9	31.8	517	.482	6.1	7.8	9.8	21.3	66.0	5.5	5.8	0.8
19	3	32.9	6.13	52.0	9.7	30.0	517	.473	6.9	5.0	10.3	24.8	60.1	4.2	9.5	0.7
20	3	32.4	7.33	48.0	12.1	34.3	517	.480	6.3	7.0	10.2	30.8	58.7	3.5	5.3	1.3
21	6	33.7	6.25	52.4	10.2	30.8	500	.445	5.4	6.3	10.2	25.2	58.9	5.1	10.3	0.4
22	4	34.6	6.80	53.2	11.7	32.5	500	.430	5.8	8.0	10.3	16.6	58.7	9.4	10.0	1.0
23	4	34.7	6.35	55.0	10.9	31.5	538	.472	5.8	5.3	9.5	20.5	66.3	4.9	6.4	0.5
24	3	35.0	6.43	54.3	11.9	34.3	517	.483	6.6	7.5	7.7	21.7	60.5	8.7	8.5	0.7
25	3	34.3	6.33	55.0	11.9	34.3	525	.473	6.6	4.8	9.6	22.0	62.8	6.0	9.5	0.3
26	3	38.0	6.53	58.0	11.1	29.0	483	.447	6.7		9.8	16.3	65.3	7.0	11.3	0.7
27	3	35.7	6.66	54.0	11.2	31.3	533	.490	6.0		8.8	24.0	59.7	5.8	8.7	1.0
28	3	33.0	5.90	58.6	11.2	33.7	540	.480	6.0		8.5	12.2	64.7	4.0	9.2	0.3
29	2	37.0	6.85	54.5	12.7	34.5	550	.552	7.3		9.4	15.5	75.0	4.0	5.5	0.0
30	2	34.5	6.40	54.0	10.4	30.0	500	.500	5.8		9.0	20.8	67.5	3.8	8.5	0.0
Cows		33.6	5.97	51.1	11.3	33.6	520	.474	6.1	6.0	7.0	30.3	51.4	8.3	9.9	0.1

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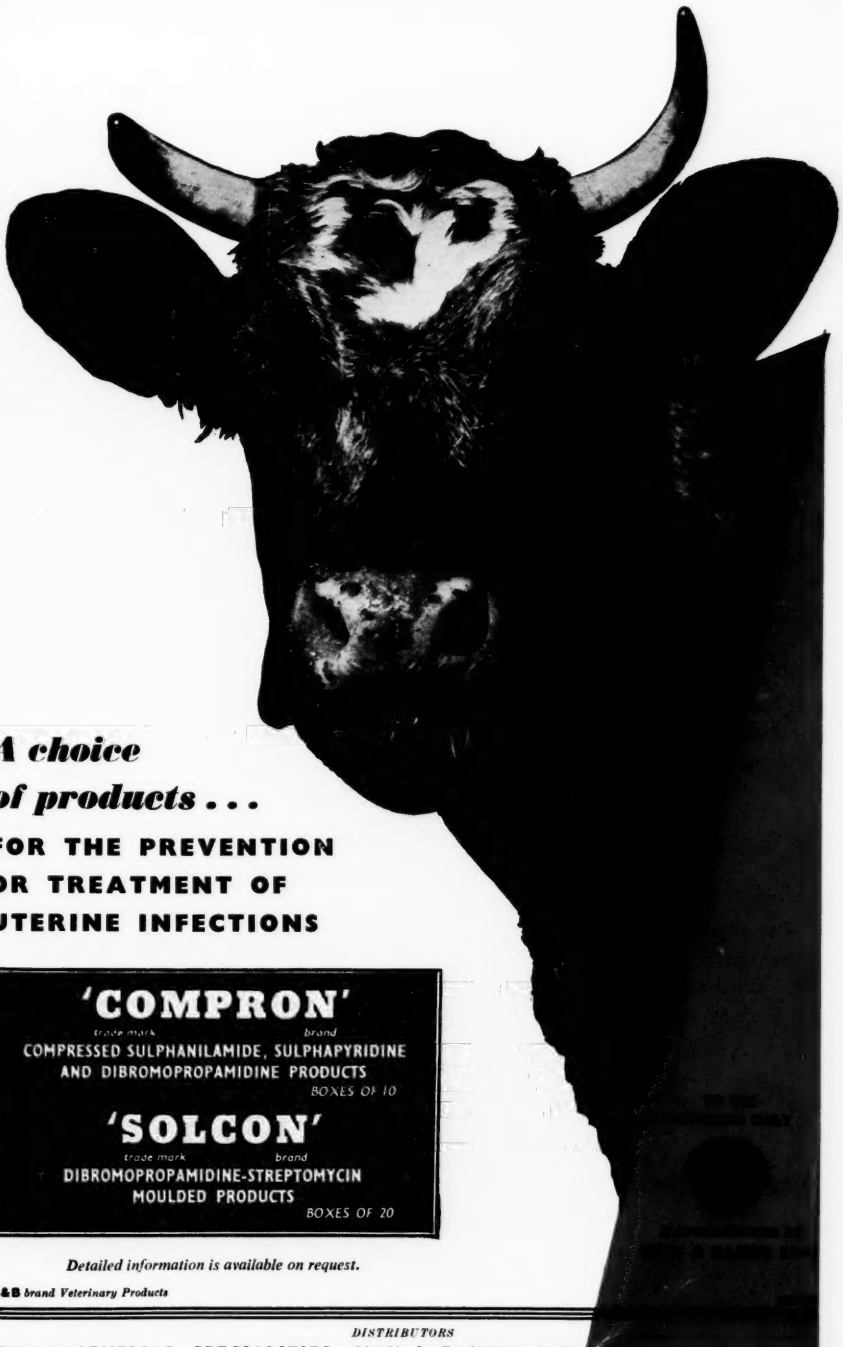

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the water-balance of the individual animal and this may account for the lack of fluctuation.

Table I shows that at birth the corpuscular size averaged 44.9 c.micra; a smaller size than that recorded for the cow. After birth the corpuscle quickly decreased in size until at 2 months the average had fallen to 30.8 c.micra. From

TABLE II

Mean Corpuscular Diameter and other Erythrocytic Dimensions of selected calves.

Age	Samples	P.C.V.	R.C.	M.C.V.	M.C.D.	M.C.Av. Skin Thickness †	Hb.	S.G. Blood	S.G. Plasma
Calf 1									
12.30 hrs.	Born								
12.45 hrs.	1	35	10.4	33.6	5.53 ± .489	1.40	10.7	1.048	1.023
15.30 hrs.	1	35	11.3	31.0	5.61 ± .406	1.25	12.2	1.050	1.025
Days 1	1	34	11.8	28.8	5.57 ± .289	1.18	11.9	1.045	1.023
5	1	33	12.8	25.6	5.35 ± .311	1.08	12.4	1.050	1.028
7	1	31	10.4	29.8	5.57 ± .298	1.22	10.7	1.050	1.028
20	1	27	9.4	26.8	5.35 ± .365	1.19	8.2	1.045	1.028
29	1	26	7.8	33.4	4.25 ± .664	2.35	8.6	1.045	1.025
43	1	26	8.2	31.7	4.59 ± .469	1.92	9.9	1.045	1.025
61	1	24	9.6	25.0	4.47 ± .463	1.59	10.9	1.042	1.025
Calf 2									
13.00 hrs	Born								
14.30 hrs	1	42	8.7	48.2	5.41 ± .409	2.10	13.2	1.047	
16.30 hrs	1	37	10.5	35.2	5.49 ± .464	1.48	12.5	1.047	
Days 1	1	35	8.0	44.0	5.57 ± .443	1.80	11.6	1.050	
3	1	26	6.2	42.0	5.33 ± .472	1.88	8.6	1.047	
5	1	35	7.3	48.0	5.28 ± .443	2.19	8.4	1.050	
Calves 3/6									
months 4	4	38	10.2	37.7	4.74 ± .416	2.14	11.7	1.053	
6	4	34	8.8	39.8	4.78 ± .409	2.22	11.2	1.053	
10	4	37	8.4	42.0	5.03 ± .396	2.10	9.9	1.051	
12	4	35	7.9	42.0	4.92 ± .362	2.21	10.4	1.054	
Cow	34	34	6.0	57.1	5.75 ± .442	2.21	11.3	1.052	(1.028)*

† Calculated from: $= \frac{MCV}{\left(\frac{MCD}{2}\right)^2} \mu$ * Wirth

this age there was a steady increase in size until the line met the level of 57 c.micra which had been recorded for the cow.

To find the best line for this steady increase, the 108 samples falling within the period of 3 months to 21 months were used to calculate a regression line. This calculation gave an average of 44 c.micra at 12 months with a regression of + 0.804 per month. The value of t was 3.5 and p = less than .001. With this regression the corpuscle by the 29th month reached the size recorded for the cow.

If the corpuscle changes in size, a change in its diameter would be expected. The few measurements made on the mean corpuscular diameter are recorded in Table II and it can be seen that over the first 20 days the M.C.D. was about 5.5 μ, falling to 4.5 μ at two months and increasing to about 5 μ at 10 or 12 months. These changes confirm the changes shown in the M.C.V. It is to be noted that the higher M.C.D. at birth was not due in either calf to the presence of macro-normocytes (Fraser, 1931) but depended on the average cell size, the largest cells having a diameter of 6.5 or 7 μ.

Compensatory Changes in Erythrocytes from 4 months to 2 years. Com-

paring the change in corpuscular size with the change in the packed cell volume over the first four months the changes are closely similar and in the figure it can be seen that at six weeks both measurements had fallen about 33 per cent; they then rose in a comparable curve until the age of four months. At four months a significant difference occurred for, although the corpuscular size continued to increase for about another two years, the packed cell volume began a roughly horizontal line at the same level as that recorded for the cow, although periods of low values occurred.

If after four months the packed cell volume remained constant as the corpuscular size increased, the logical explanation would be that there was a complementary fall in the number of erythrocytes and the figure shows that this is the case. To measure this fall in red count, and to test its validity, the regression was calculated for the 108 samples obtained between the 3rd and the 21st months. This calculation gave an average of 7.87×10^6/c.mm. for the 12th month with a regression of −-0.178 million per month. The value of t was 8 with p = less than .001. With this regression the 6 million level recorded for the cow was reached at 22 months.

If the increase in size of the erythrocyte was compensated for by a fall in number so that from the fourth month the packed cell remained constant, then it is likely that over the same period the hæmoglobin values would also give a fairly horizontal line, and it can be seen that this is the case, although until the twentieth month the values remain rather lower than the average recorded for the cow.

Changes occurring during the first four months. If during the first four months the corpuscular size and the packed cell volume fall and rise together, then a similar fall and rise would be expected in the hæmoglobin values, while in contrast the erythrocyte count should remain level.

The values for hæmoglobin in the figure show that in comparison to the P.C.V. the hæmoglobin content varied in the same direction and over the same periods, but to a lesser degree; thus the preliminary fall was about 18 per cent. The question as to whether this smaller fall was significant or whether it was due to the coincidence of several low values of different samples was examined in Table III which shows how 78 samples, which gave an average of 10.8 g., were distributed above and below this average for three different periods, and it can be seen that comparing these figures with a theoretical of 50 per cent above and 50 per cent below this line, the probability was less than p = .001. Thus the deviation from a horizontal was unlikely to be due to chance alone, and an actual fall and rise agreeing in direction and time but not in extent, with that shown by the packed cell volume can be assumed to occur.

It has been shown that there was a 33 per cent fall in packed cell volume and only about a 18 per cent fall in hæmoglobin, leaving a discrepancy of about 15 per cent. Looking at the line for the mean corpuscular hæmoglobin concentration it can be seen that this discrepancy was due to a compensatory rise in the concentration of hæmoglobin in the corpuscles, and that this rise was greatest when the packed cell volume was lowest.

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The erythrocyte count, although falling and rising over the same periods as the P.C.V., fell less than 5 per cent, and inspection of the figures for individual calves showed big variations with counts increasing for some calves and decreasing for others at each sampling; some idea of this variation can be seen from calves 1 and 2 recorded in Table II. The variation for the eleven calves sampled

TABLE III

Significance of the fall in values over the first month of life for hæmoglobin content and erythrocytic count. Compared to theoretical of fifty per cent above and fifty per cent below the average.

Period	First and second days	Remainder of first week	Remainder of first month	χ^2	p
Haemoglobin					
Above 10.8%	22	17	11	23.5	> .001
Below 10.8%	0	10	18		
Erythrocytic count					
Above 9.3	12	6.5	14	3.0	.3/.5
Below 9.3	20	10.5	15		

on the day of birth gave a mean and standard deviation of 9.8 ± 2.4 as compared to 6.0 ± 0.76 given for the cow. Tested for three periods during the first month, using the same method as used for hæmoglobin, and classifying each sample as above or below the mean of 9.3 million given by the 78 samples, it can be seen from Table III that there was no evidence that the first fall was a natural one as the data was compatible with a horizontal line.

Signs of immaturity in the erythrocytes were looked for during these early weeks but they appeared to be limited to an occasional normoblast. Normoblasts were recorded when doing the Price-Jones curves of calf No. 1 in Table II, and one or two normoblasts were seen in all films for the first week, but not in later films.

Physical Properties.

Specific Gravity. The line given in the figure for specific gravity agrees so closely with that obtained for hæmoglobin that the only comment necessary is to point out the lack of agreement between specific gravity and hæmoglobin on the day of birth.

Fragility. From the figure it can be seen that corpuscles are most resistant on the day of birth after which there is a sharp increase in fragility to reach a maximum at about four months and then a gradual increase in resistance to about two years.

The regression calculated for the 104 samples included within the 4th and 21st month showed that at 11.8 months the average fragility was 0.5 per cent NaCl with a decrease of 0.0036 per cent per month. Tested for validity $t = 6$ and $p =$ less than .001. The line joined the level of 0.474 recorded for the cow, at 20 months.

Viscosity. The line in the figure is interesting in that the changes possessed the same time periods as the erythrocytic changes and yet did not correlate with any one constituent. Thus the preliminary changes agreed with those of the

packed cell volume and after that the gentle rise from seven months disagreed with the packed cell volume but agreed with the change in size of the erythrocyte.

Clotting time. The full number of samples were not obtained for clotting time and this together with the great variation among individuals conceals any significance in the line produced by averages. The results are recorded only in Table I, and it can be seen that the figures for the first two months were higher than the succeeding ones; this was due to some individuals giving an occasional clotting time of 22 minutes or on one occasion the extraordinary value of 42 minutes.

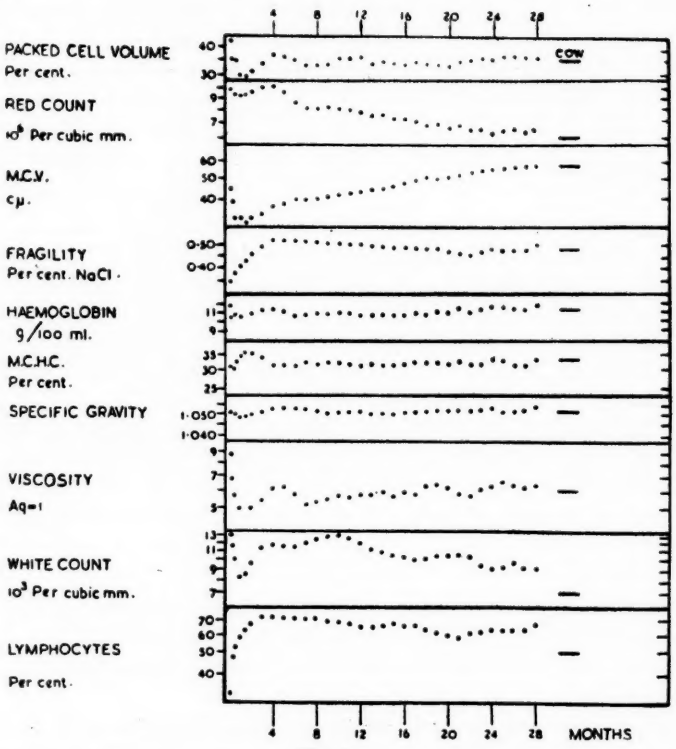

Fig. 1.
Alterations in the blood picture of calves and heifers with advancing age. From the second week onward these points are based on a three point moving average calculated from the averages given in Table I. The short black line represents the average value for cows of the same herds.

Leucocytic Picture.

The wide variations that are found in leucocytic counts in apparently healthy animals, and the many conditions, known and unknown, that affect their proportions, make it difficult to decide which changes are associated with age, and which are due to environment. The averages for all counts are recorded in Table I.

White Count. The line given by moving averages is fairly definite for the

leucocytic count and is included in the figure. It can be seen that starting at about 13,000 on the day of birth the line dropped to reach a figure of 8,000 at six weeks and then climbed to the fourth month to reach 11,500. There was then a slight rise to 12.5 by the tenth month followed by a gentle fall over the rest of the period.

Differential Count. The best line was given by the lymphocyte percentage and this is also included in the figure. It can be seen that the proportion on the day of birth was as low as 33 per cent; the line then climbed rapidly to about 71 per cent on the third month, reversing proportions with the neutrophil cells, and then slowly fell. A straight line regression on 108 samples between three months and 21 months gave a fall of 0.74 per cent each month and was highly significant ($p = 0.001$). The gradual fall in total leucocytic count over the same period shows that there was an even more definite fall in the absolute lymphocytic count.

The neutrophils fall in the same sudden manner as that in which the lymphocytic percentage increased, and the two lines crossed at about four days. Having fallen from 60 per cent on the day of birth to under 20 per cent at four months the line fluctuated presumably according to change in environment, but showed some irregular increase in harmony with the proportional decrease in lymphocytes.

Monocytes show the same preliminary fall and recovery as the total count, this fall was only from 7.5 per cent to 5 per cent followed by a rise to 8.5 per cent, but it was based on 93 samples with the rise dependant on five consecutive averages, and it did appear to be genuine. Following this there was a fall from 8.5 per cent at four months to 4.5 at 20 months.

Eosinophils remained at about 1.5 per cent for about six months and then steadily increased to about 10 per cent by 22 months. Basophils also increased in numbers at about six months and then remained at the level of about 0.7 per cent.

Discussion

Erythrocytic Picture.

The changes recorded were for the most part statistically highly significant, but the number of calves was small and before accepting these fundamental changes as natural changes associated with advancing age in all calves some confirmation from other published work would be very desirable. It is therefore very fortunate that Greatorex (1954) has recently published weekly averages for blood measurements on groups of calves of various breeds. Different calves, usually four in number, were sampled at each period; this method introduces much variation into the sampling and this may obscure the natural changes, but changes that do remain significant are strengthened owing to the larger population observed.

The most important change postulated from my results was a change in corpuscular size. This observation, that the corpuscle decreased in size until about the second month and then increased in size, is confirmed by Greatorex's figure, thus: Average size for six calves on day of birth, 67.3 c.μ. Average size for

20 calves centred on second month (7th to 11th week), 48.6 c.μ. Average size for 20 calves centred on the 50th week, 61.3 c.μ.

Canham (1930), working on South African cattle, supplies further confirmation of these changes in corpuscular size. Average M.C.V.s as supplied by his tables are as follows: Four calves on day of birth, 41 c.μ.; four calves 2 months old, 37 c.μ.; and the average for the cow, 47 c.μ.

The change second in importance was that of the red count. This count, although very variable for individual calves, kept to a high level for four months and then decreased in numbers as the corpuscles increased in size. Averages for millions per c.mm. worked from Greatorex's table for day of birth and then each two-month period are as follows: 7.4, 8.0, 7.7, 7.8, 7.0, 6.5, 6.3. It can be seen that his figures confirm the irregularity of the first few months followed by a steady fall over the last eight months. The slight fall in red count recorded in my data is not confirmed by his figures, for the average of 20 calves centred on the ninth week was 8.0 million.

Observations on the red count have been made by other workers. Fraser gives tables showing the following averages:—

> Six calves on day of birth, 7.9; four calves about one week old, 6.3; 24 calves 2 to 4 months old, 8.1; heifers, 7.2; and cows (averaged from dry cows and cows in milk), 6.2.

Canham recorded a gradual drop in red count with the increase in age of bull calves in South Africa and Mullick and Pal (1943) recorded a similar decrease in Hissar cows in India. The figures of both Fraser and Canham are in agreement with the observation that although the average figure over the first four or five months is a high one, the individual variation between calves is large, and Canham influenced by Neser (1923) suggested that much of this fluctuation was dependent on the amount of exercise taken, the active animal retaining a high hæmoglobin level; he displayed three instances in which calves had higher red counts at three or four weeks than they had shown a day or two after birth.

It has been recorded that measurements for the rise and number of corpuscles reached the same level as the cow at about two years. Only an approximation is possible for inspection shows a wide variation in individuals and the regressions disagree, for the red count reached the adult figure at 22 months and the corpuscular size reached it at 29 months, although presumably the change in size and number would end at the same time. It must also be noted that these and other changes have been treated as straight lines, although the data for the red count gave a smaller error when turned into natural logarithms, and other lines appeared straight when plotted on logarithmic paper. It was found, however, that the resulting logarithmic lines were so similar to straight lines that the tedium of calculating logarithmic lines was not justified for precision could not be expected from the small samples available.

It was shown that owing to the interplay of corpuscular size and number, the packed cell volume fell until the second month and then climbed to reach the same level as that recorded for the cow at about four months. The figures given by Greatorex give the following percentages for packed cell volume:—

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Birth, 48.5; 2 months (20 calves centred on the 9th week), 39.8; 5 months (20 calves centred on the 20th week), 44.4.

These figures agree with a preliminary fall and rise. Canham's four calves at two months showed a decrease in corpuscular volume but no fall in packed cell volume; this result might be due to some factor affecting water-balance.

Wirth records that a fall in hæmoglobin occurs during the first week of life in all animals and this first fall in calves is confirmed by Greatorex, whose average falls from 13.4 to 12.4 g./100 ml. over the first week, and Fraser who recorded a fall from 87 to 73 per cent (Tallqvist) over that period. The subsequent fall to two months and rise to four months followed by a second fall, as recorded in my data, does not quite coincide with the time intervals seen in the table given by Greatorex, and his results suggest the following periods:—

Weeks 11 to 16, average 10.8 g./100 ml.; weeks 17 to 51, average 11.6 g./100 ml.

Thomas, Okamato, Jacobsen and Moore (1954) have also recorded a gradual fall in hæmoglobin until the 30th to 70th day of life, followed by a gradual increase; there was, however, a good deal of variation in individual hæmoglobin levels. In their experimental animals seven calves, showing a mild pathological degree of anæmia at about three weeks, improved when iron was administered; no observations were made on calves that were not anæmic. Thomas *et al.* emphasise the lack of iron in milk and suggest the possibility that this is the cause of the first fall. The matter remains unproven but in view of the continued fall in the size of the erythrocyte over this period the low hæmoglobin will be considered as associated with age.

The second fall, commencing at about six months in the data given by Greatorex and in my data, probably corresponds to the very low hæmoglobin values given by Fraser for his yearling bulls at grass, and it can be assumed that the growing animal from about 6 to 20 months is, in some environments, susceptible to some degree of physiological anæmia in spite of the relatively high erythrocyte count, and that this physiological anæmia is associated with environment and not directly with age.

My finding that there was an increase in the M.C.H.C. when the packed cell volume was small at the two-month period is in agreement with the high values recorded by Greatorex for the period from the 7th to the 11th week, although in his records periods of high values also occur at later times.

Regenerative changes in my calves were limited to the presence of normoblasts and I agree with Fraser that they can occur sporadically at any age but are more frequent in young calves; Greatorex recorded five normoblasts in 26 films made from calves up to the age of four weeks. Fraser recorded reticulocytes and punctate basophilia in some of the calves examined during the first two days of life but neither Greatorex nor myself could discover punctate basophilia, and Greatorex, who stained films for reticulocytes, was unable to find them in any of the films from six calves sampled on the day of birth.

Greatorex recorded anisocytosis, particularly in the early weeks, and also the presence of "megalocytes," by which presumably he meant those macrocytes that occur in healthy blood that Fraser termed macro-normocytes. My Table II

gives no confirmation of the presence of anisocytosis in young calves and the only standard deviation that can be considered high when compared to the normal for the cow was the sample taken after about a month in Calf 1, in which, owing to the presence of numerous corpuscles only 3 micra in diameter, the S.D. became as high as .664. With this exception it can be seen that generally the standard deviations are smaller than in the cow and this is due to the rarity of corpuscles as large as 7 micra in diameter. Among the calves in Table II the only macronormocyte seen was one corpuscle of 7.5 micra seen in the film from Calf 1 taken at 61 days, and I would therefore consider them exceptional in the young calf.

Physical Properties.

No figures are available for comparison with the results for specific gravity or viscosity, but Greatorex has measured fragility, clotting time and erythrocyte sedimentation rate.

The result recorded by Greatorex for fragility was that for the first month calves had values between 0.3 and 0.35 per cent NaCl, these increased to between 0.4 and 0.45 per cent during the next four months and then remained at about 0.50 per cent up to the end of the year. This result gives confirmation to the preliminary rise recorded over the first four months in Fig. 1 but does not confirm the slow but highly significant fall over the following 16 months.

Clotting time fluctuated in both Greatorex's and my investigations. Greatorex recorded that the time was much slower between the 20th and 35th week than either before or after this period, but in my results the clotting time was slower during the first two months. The fact that the most impressive peaks in our fluctuations did not agree in time period suggests that in both cases the peaks were fortuitous or were the results of unknown causes not associated with age. Greatorex used the same technique as I did and the modal time recorded in his distribution table for 227 calves was six minutes, the time that I recorded as the average for the cow, but the highest clotting time he recorded was 11 minutes as compared with my occasional measurement of 22 minutes and the exceptional measurement of 42 minutes. This exceptional measurement was made in a calf two days old and does not appear due to an error in technique as subsequent times also appeared somewhat abnormal. The actual times in minutes were as follows: Day of birth, 9; 1 day, 22; 4 days, 42; 6 days, 15; 13 days, 8; 20 days, 9; 4 weeks, 3½; 6 weeks, 14; and 8 weeks, 19. These results suggest that although in general the clotting times for cows and calves are similar, some healthy calves can show abnormally long clotting times.

The erythrocyte sedimentation rate recorded by Greatorex showed a very obvious negative correlation with the hæmoglobin values which would mask any small change associated with age, but, as the modal figure in the distribution table for 233 calves was 3 mm. in 24 hours, and as Bunce (1954), using the same technique, showed an average of 2.96 mm. for six cows, there is no evidence to suggest that the E.S.R. in the calf is different from the cow.

Greatorex received the impression that the blood platelets in calves were larger than in adult cattle; he recorded outside limits as from 150,000 to 525,000 per c.mm. with a common range of from 250,000 to 400,000. He observed no

changes associated with age and his common range is in good agreement with the average of 350,000 per c.mm. recorded for the cow when using the same technique (Holman, 1955). It can be assumed, therefore, that the same standards are applicable to both cow and calf.

Leucocytic Picture.

It was recorded that the average leucocyte count started at the high figure of 13,000 c.mm. and then showed a definite fall. Neither Fraser nor Greatorex record a similar occurrence and their averages for six calves taken on the day of birth were 10,000 and 8,700 per c.mm. respectively, with no sign of a fall in the weekly averages recorded by Greatorex. My figures show great variation in samples from individual calves, thus two calves with counts of 18,000 on the day of birth showed counts of less than 10,000 on the following day, conversely one calf with a count of 9,800 on the day of birth gave a count of 17,000 the next day, and in another calf bled on the day of birth the highest count (13,500) was in the fifth sample, taken a fortnight after birth. Thus individual calves show no definite trend and as other workers offer no support either for the initial high values or their subsequent fall, the curve shown in the figure cannot be accepted as being associated with age.

The changeover in the proportion of lymphocytes and neutrophils has already been noted by Fraser, and Wirth (1950) quotes Jedlicka as recording its occurrence at some time between the second and fifth day. The gradual fall from the fourth month onward receives support from the figures given by Greatorex which show the average between the 5th and 12th weeks was 72 per cent lymphocytes compared with an average of 68 per cent over the last eight weeks.

The increase in eosinophils associated with age has already been recorded by Fraser and Greatorex, and the assumption that this increase is due to parasitism led Greatorex to carry out worm egg counts on the fæces of all calves showing a count of over 11 per cent eosinophils, but he was unable to demonstrate that eggs were present, and thus there is no reason to reject the idea that the increase in eosinophils is associated with age.

Monocytes are too variable in number for an interpretation of their fluctuation and even my average of 6.1 per cent from the 216 samples from 22 calves lies in contrast to the value of 3.2 per cent obtained from 228 films examined by Greatorex.

Clinical Standards.

A paper on the blood picture of an animal is of little value if it does not attempt to help other workers by trying to set standards, even if subsequent work is likely to bring some readjustment of the recorded values. It was seen from the discussion that not all the changes recorded in the results could be accepted as being determined by age and therefore a set of modified values based on the recorded values for the cow and showing the changes believed to be associated with age are recorded in Table IV.

In this table the results for the hæmoglobin and for the erythrocyte counts are comparable with those recorded by Greatorex, but his figures for the packed

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cell volume are somewhat higher than those recorded; his value at four months would be about 42 per cent with the previous values altered in proportion. With regard to physical properties the figures for fragility do not disagree with the results given by Greatorex; the clotting time after the comparison of our results has been recorded as remaining unchanged; and the specific

TABLE IV

Modified Table of Changes Associated with Age.

	Birth	One Week	Months 2	4	24
Haemoglobin g/100ml.	13	11	10.5	11	11
Packed cell volume per cent.	42	35	32	35	35
Erythrocyte count 10^6/c.mm.	8	8	8	8	6
Mean Corpuscular Volume c. micra	52	44	40	44	57
Mean Corpuscular Haemoglobin concentration per cent.	31	31	32	31	31
Mean Corpuscular Diameter c. micra	5.5	5.3	4.5	4.7	5.7
Fragility per cent. NaCl.	.35	.36	.44	.53	.47
Clotting time: minutes.	6	6	6	6	6
Specific Gravity	(Positive correlation with $\frac{Hb}{Hc}$.)				
Erythrocyte Sedimentation Rate (Greatorex)	(Negative correlation with $\frac{Hb}{Hc}$.)				
Viscosity. Aq. = 1.	8.7	6.7	4.3	6.0	6.0
Leucocyte count	9	9	9	9	9
Lymphocyte percentage	33	40	68	72	52
Eosinophils percentage	0.2	1	2	3	9
Platelets (Greatorex) 10^3/c.mm.	350	350	350	350	350

To read intermediate periods transfer these figures to graph paper and join the points by straight lines; for slightly greater accuracy plot on semi-logarithmic paper.

gravity and the erythrocyte sedimentation rate has been seen to be dependent on the hæmoglobin figures. The viscosity measurements, in which results are so dependent on method, are quite unconfirmed. The leucocyte count has been recorded as unchanged as shown by the larger population sampled by Greatorex, but the lymphocytes are recorded as showing the changes recorded in the figure, for which the first rise was confirmed by other authors and the subsequent slow fall shown to be statistically significant. The figures for eosinophils are also partly based on the figures given by Greatorex.

Coming now to the criteria for pathological states, the term pathological anæmia was restricted in the cow to values falling below 8 g./100 ml. As the hæmoglobin values in calves are similar to those in cows the same criterion might be acceptable. Applying this standard to the 216 samples from my 22 calves, none could be classified as anæmic, but for the figures given by Greatorex for 232 calves, six values were excluded. These values were 7.7, 7.4, 7.2, 6.3, 5.5 and 4.6 and they were distributed sporadically throughout the year. The weekly averages in which these averages are included suggest that these figures are abnormal and in view of the susceptibility of young animals to anæmia the proportion of 2.6 per cent given by these figures does not seem unduly high and the criterion of 8 g./100 ml. can be regarded as a realistic one.

In the cow the definition of hypochromia was based on the recording of a mean corpuscular hæmoglobin concentration of less than 24 per cent and it

was suggested that values between 15 and 18 per cent would indicate hypochromia in a moderate degree. It does not seem logical to formulate one criterion for hypochromia in the cow and another for the calf, and yet if this criterion is applied to the figures for my calves—after they have been corrected for shrinkage—then half the 22 calves show a slight hypochromia in one sample or another; the lowest figure being 22 per cent, and also, out of the 233 calves sampled by Greatorex, 11 show hypochromia by this standard; of these two show moderate hypochromia and two, with values 13 and 14 per cent, show a severe hypochromia; the hæmoglobin values for these two were 4.6 and 7.2 g./100 ml. respectively.

Five calves sampled by Greatorex and all the 11 calves in my data classified as showing hypochromia did not have levels of hæmoglobin that were low enough to indicate anæmia. Whitby and Britton link hypochromia with iron deficiency so firmly that they speak of the M.C.H.C. as being the key to iron therapy, so that from the above figures it would seen that in calves there is a stage in this iron deficiency in which there may be a moderate degree of hypochromia without a deficiency in the concentration of hæmoglobin in the blood; if this is accepted then it might be useful to term this condition "simple hypochromia" to differentiate it from hypochromic anæmia in which hæmoglobin values fall below the criterion of 8 g./100 ml.

The criterion for anhydræmia in the cow was the measurement of a packed cell volume of over 50 per cent. Applying this criterion to the corrected values of my calves, two calves exhibited a slight anhydræmia on the day of birth and one also on the succeeding day. Among the calves sampled by Greatorex, 6 values exceed this criterion and with one exception these occur in the first four or five months, confirming the greater variability of the erythrocyte count over this period. These results suggest that providing it is noted that the fluctuation in water balance is greater in the first four or five months of life, there does not seem to be any need for a separate criterion for the measurement of anhydræmia.

In the previous paper it was stressed that leucocytosis could be physiological as well as pathological in origin, and the condition was arbitrarily defined as occurring where the leucocyte count was over 13 x 10^3/c.mm. In the calf a slight degree of leucocytosis would appear to be more common than in the adult animal, and my figures show two values as high as 18,000 on the day of birth; Fraser shows figures 14,000 and 15,000 for two of his 37 calves, and Greatorex shows three calves at that level with five other calves on the borderline at between 13,000 and 14,000. Low values are less common than in the adult and my lowest value was 5,000. Fraser records only one calf at 4,000 but others at 6,000, and the lowest figures given by Greatorex was 4,500 with several calves between 6,000 and 7,000. The values of over 13,000 for leucocytosis and under 4,000 for a leucopenia are therefore acceptable but it should be remembered that in calves high figures occur more often and figures below 6,000 less frequently than in cows.

Summary

Changes were recorded in the blood picture of calves and heifers. Where

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possible the results were modified according to the results of other authors, and acceptable figures summarising the changes associated with the age are recorded in a table.

It was found that the erythrocyte averaged about 52 c.micra at birth but rapidly decreased in size over the first week and then decreased more slowly to average 40 c.micra at about two months. After two months the corpuscle slowly increased in size to reach 57 c.micra at the end of two years. During the first four months the packed cell volume and the hæmoglobin fell and rose again in harmony with the change in corpuscular size while the erythrocyte count remained constant, although showing great fluctuation in the individual animal. At four months the packed cell volume and hæmoglobin remained fairly constant and the erythrocyte count fell as the corpuscular size increased.

The fragility of the erythrocyte increased rapidly over the first four months and then became slowly more resistant until at the end of two years it was similar in degree to the cow. Viscosity fell sharply over the first two months and then began a slow irregular rise to reach the figure recorded by the cow at two years.

The lymphocytes represented about 33 per cent of the differential count at birth and the proportion rose to 72 per cent at four months after which there was a slow fall which suggested they would reach the proportion recorded for the cow at the end of two years. Eosinophils represented less than one per cent at birth and rose irregularly.

Clotting times and platelet counts appear to agree with adult standards except that an occasional calf showed a very slow coagulation time and specific gravity shows a positive correlation with hæmoglobin.

The clinical standards set for cows appear to be applicable to calves but the following observations are of interest. Anæmia is more likely to occur in the calf and there is a tendency for calves to become anæmic at some period between 6 and 18 months; this change appears to be associated with environment and not with age. Calves can show a slight or moderate degree of hypochromia even when the hæmoglobin level in the blood is too high to record as anæmia; the term "simple hypochromia" has been suggested to describe this state. During the first four or five months the water balance appears to fluctuate widely and values indicating anhydræmia in the adult are sometimes observed and there is wide variation in serial erythrocyte counts. Although the average leucocyte count is similar to the adult, a higher proportion of calves show physiological leucocytosis and few calves show counts of under 6 x 10^3/c.mm.

REFERENCES

Bunce, Shirley, A. (1954): *Brit. vet. J., 110,* 322.
Canham, A. S. (1930): 16th Report, Div. Vet. Services. Pretoria: The Government Printer.
Fraser, A. C. (1930): *Rep. Dir. Inst. anim. Path., Camb., 1,* 114.
Greatorex, J. C. (1954): *Brit. vet. J., 110,* 120.
Holman, H. H. (1955): *Brit. vet. J., 111,* 440.
Mullick, D. N., and Pal, A. K. (1943): *Indian J. vet. Sci., 13,* 146.
Neser, G. P. (1923): 9/10 Report Div. vet. Res. Pretoria: Governmennt printing office.
Thomas, J. W., Okamoto, M., Jacobsen, W. C., and Moore, L. A. (1954): *J. Dairy Sci., 37,* 805.
Whitby, E. H. L., and Britton, C. J. C. (1939): "Disorders of the Blood," 3rd ed. London: Churchill.
Wirth, D. (1950): "Grundlagen einer Klinischen Hæmatologie der Haustiere," 2nd ed. Wien/Innsbruck: Urban und Schwarzenberg.

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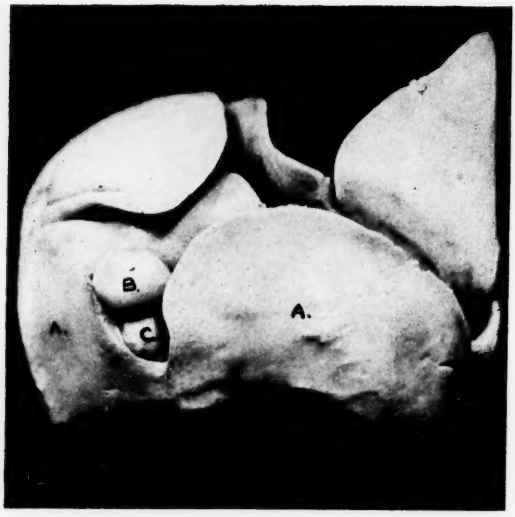

Fig. 1
A. Central lobe. B. Gall bladder. C. Secondary diverticulum of gall bladder.

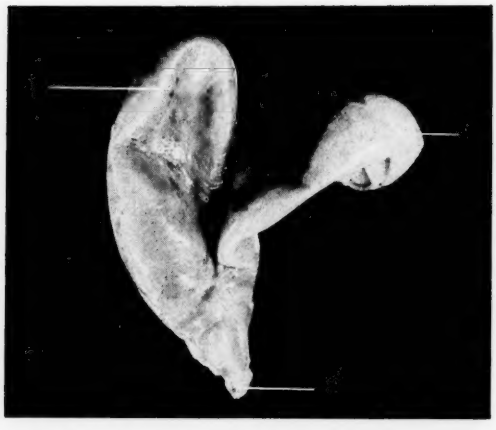

Fig. 2
A. Gall bladder. B. Secondary diverticulum. C. Cystic duct.

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A SECONDARY DIVERTICULUM OF THE GALL-BLADDER OF A CAT

By P. G. D. MORRIS, M.A., M.R.C.V.S.

Department of Anatomy, Royal (Dick) School of Veterinary Studies, University of Edinburgh

THE abnormality here described was noted during the dissection of a cat in the Department of Materia Medica in the University of Edinburgh and the specimen was passed on to me. On making a survey of the literature it appears that abnormalities of the gall-bladder of the cat do occur fairly often but opinions vary as to the frequency, and the following figures of occurrence are given: 1 in 8, Boyden (1926); 1 in 200, Bartlet (1951); 1 in 900, Gribble (1950). These figures are all based on work carried out on cats in the United States of America and quoted by Mann and Fratter (1952). The Department of Pathology in the Edinburgh Veterinary College has post-mortemed 696 cats during the years 1940-1953 and no cases of a secondary diverticulum or accessory gall-bladder have been recorded, and the following case is, as far as I know, the first to be noted in this Department.

According to Boyden, who carried out very extensive studies of gall-bladder anomalies, "the commonest type of variation in the cat is the partially subdivided gall-bladder. It exhibits several patterns, depending upon the number of lobes into which the biliary vesicle is divided and upon the depth and position of the intervening clefts." Boyden further says this subdivided form usually produces a bi-lobed saccule, but tri-lobed and exceptionally quadri-lobed saccules occur. While the double biliary vesicle is seen in cats, it is very rare and its origin is not easily explained. It is suggested that this type develops not as a splitting of the primary vesicle but by the growth of an independent bladder from a diverticulum of the original cystic duct.

Description

From a superficial examination of the specimen it looked as if there might be two gall-bladders present (Fig. 1). The lobation of the liver was carefully

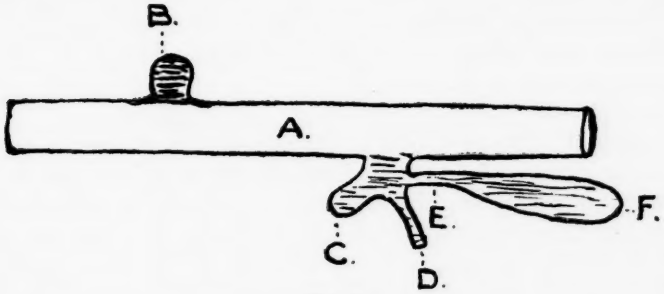

Fig. 3

Diagram to show development of gall bladder and ducts. A. Fore gut. B. Dorsal pancreas. C. Ventral pancreas. D. Hepatic duct. E. Cystic duct. F. Gall bladder.

examined and found to be normal. When the lobes were removed from round about the gall-bladder it could be seen that the sac was distinctly bi-lobed but it was not yet possible to see if two separate gall-bladders each with a cystic duct were present. Further, dissection revealed that only one cystic duct was present, therefore it was concluded that only one gall-bladder was present (Fig. 2). If there had been duplication of the organ two cystic ducts would have been present, for in the normal way the gall-bladder and its duct develop as a special off-shoot from the original hepatic diverticulum (Fig. 3). The main bladder measured 3.4 cm. in length with a maximum diameter of 1 cm. The diverticulum measured 2.4 cm. in length with a maximum diameter of .8 cm. (measurement after fixation with Formol). Therefore there is no doubt that the additional saccule present in the specimen here described is a secondary diverticulum from the original hepatic diverticulum.

My thanks are due to Dr. M. Vogt, F.R.S., for passing the specimen on to me and to Mr. R. S. Hood, A.I.B.P., A.I.S.T., for the photographs.

REFERENCES

Boyden, E. A. (1926): *Amer. J. Anat., 38,* 177.
Bartlet, L. M. (1951): *Anat. Rec., 109,* 715.
Gribble, L. R. (1950): "Comparative Anatomy Laboratory Manual." Philadelphia: Blakeston Co.
Mann, P. H., and Fratter, I. (1952): *Cornell Vet., 42.*

OBSERVATIONS ON THE PRESENCE OF A HÆMOLYSIN IN BOVINE SEMEN

By P. G. MILLAR, M.R.C.V.S.
Ministry of Agriculture, Weybridge, Surrey.

WHILE carrying out complement-fixation tests on semen from bulls during the course of a virus investigation, it was noted that certain samples of seminal plasma hæmolysed the red blood cells and that others did not cause lysis. This observation was sufficiently interesting to warrant further investigation and, as a preliminary step, known dilutions of seminal plasma were titrated against varying suspension of R.B.C's. according to the following technique:

Materials.

(*a*) Defibrinated, washed and packed, bovine red blood cells.
(*b*) Bovine seminal plasma (obtained by centrifugation).
(*c*) 0.85 per cent saline solution.

Method.

The seminal fluid was diluted at the rate of 1 : 4 of saline and added in 0.25 ml. amounts to six blood tubes. To these were added 0.25 ml. of saline.

The washed red blood cells were suspended in saline to give six dilutions, namely, 4 per cent, 2 per cent, 1 per cent, 0.5 per cent, 0.25 per cent and 0.125 per cent. To each tube was added 0.25 ml. of the appropriate red blood cell dilutions. After shaking, the racks were allowed to stand on the bench at room temperature. Readings were made after one hour. In further tests the 0.25 ml. of saline was replaced by serum.

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Reading.

Tubes showing complete hæmolysis, with no deposition of red blood cells, were graded 4. Hæmolysis with 25 per cent deposition was grade 3. Slight hæmolysis with 50 per cent deposition was graded 2, and very faint hæmolysis with large deposition, 1. Tubes showing no hæmolysis and with a total deposition of the red blood cells were graded 0.

To gain an impression of the strength of the hæmolysin present in seminal plasma a titration was carried out using a doubling dilution technique. This

Example of Controlled Test.

Seminal Plasma 1/4	Saline					
	Bovine R. B. C. Dilution %					
	4	2	1	0.5	0.25	0.125
Unknown	tr	1	2	3	4	4
Control Positive	4	4	4	4	4	4
Control Negative	0	0	0	0	0	0
Saline	0	0	0	0	0	0

gave a range of 1 : 2 to 1 : 2048. The seminal plasma dilutions were titrated against three dilution rates of red blood cells (0.125 per cent, 0.25 per cent and 0.5 per cent). It was found that there was complete hæmolysis of 0.125 per cent blood cells throughout the whole range of seminal plasma dilutions. In the presence of 0.25 per cent red blood cells, complete hæmolysis occurred up to 1 : 128 and in decreasing amounts to 1 : 2048. In the presence of 0.5 per cent red blood cells hæmolysis was complete at 1 : 32, decreasing to nil at 1 : 256.

A routine test was then made on seventeen samples of seminal plasma from eleven bulls. These were tested in dilutions varying from 1/5 to 1/160 against a constant dilution of 0.25 per cent bovine R.B.C. in suspension. It was found that hæmolysins were present in the ejaculates of three of these bulls but in varying strengths. In order to confirm that variations in hæmolytic activity did occur between samples, further titrations were carried out on seminal plasmas obtained from field bulls.

A further series of tests were carried out on samples of seminal plasma known to be hæmolytic. The titrations revealed that the end titre varied between different samples, re-affirming that the degree of hæmolytic activity was not a constant. It was appreciated, however, that red blood cells may be lysed *in vitro* by exposure to factors such as the presence of toxins, extremes of hydrogen-ion concentration, osmotic tension and heat. Thus it was desirable to evaluate the significance of such factors under our conditions of examination. For this purpose, twenty-four samples of seminal plasma were employed from various bulls, some samples having been stored at —20° C. for varying periods of time up to ten months. The *pH* values were estimated by use of a colour indicator and the findings showed that these values varied from 4.0 to 7.0. Tube tests showed that these *pH* values were not related to the hæmolytic

properties of the samples. The average *pH* reading of the hæmolytic samples was 6.3 while that of the non-hæmolytic samples was 6.03, revealing no significent difference. It was also evident that the *pH* values were not dependant upon the period of storage of the samples. The fact that all the dilutions were made in an isotonic saline solution appeared to eliminate variation of osmotic tension as a factor inducing hæmolysis. As every care was taken to avoid adverse conditions of temperature and other mechanical factors, it would seem that such could be ignored as active agents.

In an attempt to assess whether a virus might be involved and demonstrable by hæmolytic activity when present in a fluid medium, samples of chorio-allantoic fluid were collected from embryonated hen's eggs, both normal and experimentally inoculated with infected semen. These samples were found to be non-hæmolytic and it was felt that the hæmolytic factor in semen was unlikely to be of a viral nature.

To consider the possibility that a bacterial toxin might be present in hæmolytic samples of seminal plasma, cultures were made on blood agar plates from eleven hæmolytic and fifteen non-hæmolytic samples of semen and incubated aerobically at 37° C. These samples had been stored at —20° C. for varying periods of time. Following incubation, hæmolytic colonies were present on most plates, bearing no relationship to the hæmolytic or non-hæmolytic reactions shown by the tube tests. Known negative samples were inoculated with a beta-hæmolytic bacillus, *Proteus,* which had been obtained from the culture of a hæmolytic semen sample.

The inoculated sample was incubated at 37° C. for one hour and then stored at —20° C. for 48 hours. Another portion of the same non-hæmolytic sample of seminal plasma was inoculated with a similar beta-hæmolytic spreading organism which had been obtained from a semen culture of negative significance. Controlled hæmolysin tests were carried out on these two samples, but they continued to be negative and to reveal no hæmolytic tendency. These were incubated for a further 24 hours at 37° C. and then stored at —20° C. At the same time, several non-hæmolytic incubated samples were also examined for hæmolytic properties. They revealed that the two inoculated samples had become strongly hæmolytic, even to the extent of discolouration. The non-hæmolytic samples which had been incubated at the same time had also become hæmolytic but the type of hæmolysis resembled that usually seen, in contra-distinction to that found in artificially-contaminated samples where discolouration accompanied the hæmolysis. It was felt that the strong hæmolytic factor and tendency to discolouration might be attributed to a bacterial growth in excess of that encountered in normal samples of semen. It was found that filtration of these hæmolytic samples through a Seitz EK pad did not in any way diminish the hæmolytic properties of the filtrate.

On one day nine semen samples were collected from one bull and seven semen samples from another. These ejaculates were dealt with individually and subjected to varying procedures in an attempt to elucidate the origin of the hæmolysin. These included centrifugation, passage through a Seitz EK filter,

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incubation at 37° C. for twenty-four hours, also repeated rapid freezing at —80° C. and thawing at 37° C. The results were as follows:—

A. Filtration through the Seitz asbestos pad turned non-hæmolytic seminal plasma hæmolytic.

B. When non-hæmolytic samples of unfiltered material were incubated at 37° C. for twenty-four hours they became hæmolytic to the same degree as the filtered.

C. Freeze-thawing of spermatozoa, which had been washed and resuspended in saline did not cause disruption of the germ cells or render the saline hæmolytic. It was also shown that subsequent filtration of this saline did not turn it hæmolytic.

D. The freezing of non-hæmolytic seminal plasma prior to incubation did not prevent the subsequent production of hæmolytic activity.

E. When seminal plasma known to be non-hæmolytic was left on the bench at room temperature for 48 hours it became hæmolytic, confirming previous observations.

The results of previous tests had indicated that the first ejaculate might contain a hæmolytic substance in greater degree than subsequent ejaculates from a bull on one day. This was checked by the following series of tests.

(*a*) Over a period of four days, thirteen samples of semen were collected from one bull. These ejaculates were centrifuged and frozen immediately after collection and stored at —20° C. until the hæmolytic tests were carried out three days after the last date of collection. The samples of seminal plasma were then diluted 1 : 4 in physiological saline at room temperature. These samples were not subjected to filtration. The findings were that the first ejaculate on any one day was hæmolytic, the others being non-hæmolytic. This finding was further checked by carrying out a similar experiment on the ejaculates collected from another bull. Over a period of three days ten samples of ejaculate were collected and treated as before and it was found that only the first ejaculate on each day was hæmolytic.

(*b*) From a bull of known satisfactory fertility, three ejaculates were collected within a space of a very few minutes. They were examined on the same day and it was found that although the first ejaculate was hæmolytic, the other two were non-hæmolytic. It appeared from this and previous observations that although certain bulls might be more likely to yield hæmolytic semen than others, it was possible that the first ejaculate from a fertile bull might be more hæmolytic than subsequent ejaculates on any one day.

(*c*) On one occasion three semen samples were collected from a young, previously unused bull and it was found that each of these ejaculates was strongly hæmolytic immediately after collection.

It seemed desirable to determine whether spermatozoa played any part in the production of the hæmolytic factor.

Four samples of semen were selected, two of them known to be hæmolytic, the other two non-hæmolytic. The hæmolytic samples were the first ejaculates

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from two bulls on one day. The non-hæmolytic ejaculates were the subsequent ejaculates from the same bulls. Each sample was centrifuged and the deposited spermatozoa used for a series of tests. The supernatants were tested in a further series.

(*a*) Following centrifugation, the spermatozoon content of each ejaculate was washed and centrifuged three times in sterile physiological saline. As physical methods of disruption had proved ineffective, the spermatozoa were then resuspended in 5 ml. of sterile physiological saline and incubated at 37° C. for sixteen hours in the presence of 5 mg. of Trypsin. A further 10 mg. of Trypsin were added because breakage of the spermatozoa had proceeded only to the stage of separation of the tails from the heads. Incubation was continued for a further twenty-four hours which yielded almost complete disintegration, with the formation of a gel. The material resulting from both the hæmolytic and non-hæmolytlic samples was tested for hæmolytic activity and proved negative.

(*b*) To each of the samples of seminal fluid was added four times its volume of a half-saturated solution of $(NH_4)_2SO_4$. The resultant precipitates were removed by centrifugation at 3,500 r.p.m. for twenty minutes. The supernatant fluid from each sample was then dialysed at room temperature against distilled water for twenty-four hours with three changes of distilled water to remove $(NH_4)_2SO_4$. The samples were then dialysed for a further twenty-four hours with three changes against physiological saline to recover isotonicity. As a control to the dialysing technique, a sample of physiological saline plus four volumes of a half-saturated $(NH_4)_2SO_4$ solution received similar treatment.

It was found that the hæmolytic samples of seminal plasma remained hæmolytic to the original degree and that the non-hæmolytic samples became hæmolytic. The fact that the control sample, which did not contain seminal plasma, remained non-hæmolytic suggested that the essential changes were relative to the seminal plasma. As these samples under test necessarily remained at room temperature for forty-eight hours it could be expected that the non-hæmolytic samples of seminal plasma would acquire hæmolytic properties in accordance with previous observations.

(*c*) The precipitates from the seminal plasma resulting from the addition of $(NH_4)_2SO_4$, were dissolved in distilled water and dialysed against two changes of distilled water for eighteen hours to remove traces of $(NH_4)_2SO_4$. The resultant solutions were then dialysed against physiological saline for twenty-four hours, three changes of saline being made with the intention of recovering isotonicity. Hæmolytic tests were then carried out and it was found that each of the four samples was hæmolytic to a degree similar to that noticed in (*b*). It thus appeared that the $(NH_4)_2SO_4$ had precipitated a proportion of the hæmolytic factor, and that some may have been attached to a globulin so that hæmolytic activity was demonstrable in both (*b*) and (*c*).

Seminal plasma (1 ml.) from one of the known positive samples was diluted 1 : 4 with physiological saline and dialysed against 4 ml. of physiological saline for forty-eight hours at 5° C. It was found that the saline became hæmolytic

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to the same degree as the seminal plasma and that the hæmolytic factor had passed through the dialysing membrane.

In order to improve, if possible, the sensitivity of the tube tests, titrations were carried out to determine whether the red blood cells of other species would lyse more readily. Red blood cells were obtained from the cow, goat, dog and horse by collecting blood directly into bottles, each containing a number of glass beads; clotting being prevented by continued shaking. The blood was then added to a greater volume of physiological saline, centrifuged and the cells resuspended with repeated washing, until the supernatant remained clear. The packed blood cells were then suspended in isotonic physiological saline in known concentration. Varying dilutions of these red blood cells were titrated against samples of seminal plasma which previously had been shown to be either hæmolytic or non-hæmolytic and stored at —20° C. The plasma was diluted 1 : 4 and used in 0.25 ml. amounts. Although comparable results were obtainable with the red blood cells of most species, the most clear-cut results appeared to be yielded by those of bovine origin.

At an early stage of the laboratory investigation, while still thinking along virological lines, attempts were made to determine whether there was any antigen-antibody reaction between hæmolytic samples of seminal plasma and blood serum from normal, infected and recovered heifers, associated with our transmission experiments. It was found that not only was there no specific reaction but that all the serum samples tested completely inhibited the hæmolytic properties of the seminal plasma. The above findings were checked by using other samples of seminal plasma and it was confirmed that all hæmolytic activities were inhibited and that the red blood cells were protected by the blood serum. Further tests were carried out employing other samples of semen and the findings confirmed the above results.

When serum from a heifer was titrated against a dilution of 1 : 4 of known hæmolytic and non-hæmolytic samples of seminal plasma in the presence of an 0.25 per cent suspension of washed, defibrinated, bovine, red blood cells, it was found that inhibition occurred only in low blood serum dilution rates, i.e., at 1 : 1 to 1 : 32 approximately. On the other hand, the hæmolytic factor in the seminal plasma was still demonstrable in dilutions greater than 1 : 2,000.

When blood serum and seminal plasma were mixed together in equal volumes and then diluted to varying degrees before the red blood cells were added, it was found that the hæmolytic activity was inhibited in low dilution rates. On the other hand, when the dilution rate of the mixture was progressively increased the inhibitor diluted out more rapidly than the hæmolysin which strengthened the impression that the inhibitory action of the blood serum was purely mechanical.

However, when equal volumes of hæmolytic seminal plasma and blood serum were mixed and stored overnight at —20° C. it was found that hæmolysis was completely inhibited and that there was no evidence of any particular factor, hæmolytic or inhibitory, diluting out. It seemed that complete neutralisation had occurred during the storage period.

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Bovine blood plasma, stored at —20° C. for two years, inhibited hæmolysis in dilutions up to 1 : 128. The same blood plasma, heated at 56° C. for periods varying from fifteen minutes to eleven hours, remained inhibitory in dilutions up to 1 : 128.

Confirmation that this inhibitory activity was non-specific was given by the fact that inhibition was provided by all types of sera tested, as follows :—

Goat blood serum inhibited hæmolysis in dilutions up to 1 : 256.

Horse serum inhibited hæmolysis in dilutions up to 1 : 256.

Rabbitt serum inhibited hæmolysis in dilutions up to 1 : 256.

Pig serum inhibited hæmolysis in dilutions up to 1 : 128.

Guinea-pig serum,, freeze-dried, inhibited hæmolysis in dilutions up to 1 : 64.

Dog serum, freeze-dried, inhibited hæmolysis in dilutions up to 1 : 256.

Thus it would appear that the inhibitory activities of blood serum are not diminished by exposure to a temperature of 56° C. or by freeze-drying.

The dilution rate of the blood serum which would inhibit hæmolysis was shown by a series of tests to be directly related to the dilation rate of the seminal plasma as well as to the strength of the hæmolytic factor carried by it.

A series of tests were carried out in an attempt to define which fractions of blood serum were most strongly inhibitory to hæmolysis. Serum from a heifer was subjected to the following treatments :—

(*a*) Filtration through a Seitz EK asbestos pad.

(*b*) To one volume of serum was added four volumes of a half-saturated solution of $(NH_4)_2SO_4$. The resultant precipitate was removed by centrifugation. The supernatant was then dialysed for twenty-four hours against distilled water at 5° C., to remove $(NH_4)_2SO_4$, with four changes of water. It was dialysed for a further forty-eight hours against physiological saline to recover isotonicity.

(*c*) A sample of serum was treated as in (*b*) except that the precipitate was removed by filter paper and not by centrifugation.

(*d*) The precipitated globulins from (*b*) and (*c*) were resuspended in distilled water to their original volumes and again dialysed against distilled water at 5° C. for twenty-four hours, with four changes of water, to remove $(NH_4)_2SO_4$. Further dialysation was then carried out against physiological saline for forty-eight hours at 5° C.

(*e*) One volume of serum was dialysed against two volumes of physiological saline for seventy-two hours at 5° C.

Samples of the original blood serum and each of the resultants from the above techniques were titrated against the pooled hæmolytic and pooled non-hæmolytic samples of seminal plasma. It was found that : —

(*i*) The untreated blood serum inhibited hæmolysis in each case.

(*ii*) The Seitz-filtered blood serum inhibited hæmolysis in each case.

(*iii*) The centrifuged supernatant (*b*) inhibited the hæmolytic activity of the samples.

(*iv*) The paper-filtered supernatant (*c*) showed inhibitory powers similar to those of (*b*).

(*v*) The precipitated protein (*d*) revealed no inhibitory activities.

(*vi*) The dialysed serum (*e*) inhibited hæmolysis.

(*vii*) The saline showed no inhibitory activity.

This indicated that the inhibitory activity is not removed by Seitz filtration nor by removal of the globulin fraction by precipitation with $(NH_4)_2SO_4$. The inhibitory factor is not dialysable. It would appear, therefore, that the action of the blood serum is largely that of a protective colloid to the red blood cells.

A number of samples of seminal plasma were pooled and filtered with the intention of obtaining a hæmolytic, stock, controlled sample which could be standardised. An amount of this pooled seminal plasma was diluted with physiological saline in a range of doubling dilutions, from 1 : 4 to 1 : 8,192, and tested against a 0.25 per cent suspension of bovine red blood cells. Complete hæmolysis occurred in the range up to and including a dilution of 1 : 512, decreasing to partial at 1 : 2,048. In equal volume, blood serum at 1 : 4 inhibited hæmolysis throughout the whole range of seminal plasma dilutions. However, when the seminal plasma dilution was maintained at a constant 1 : 4 and titrated against blood serum in a dilution range 1 : 4 to 1 : 8192, blood serum was capable of inhibition in dilutions up to only 1 : 32. The inhibitory capacity decreased in dilutions up to 1 : 256, at which point the inhibition was nil and the hæmolysis complete.

The stock dilution of seminal plasma, 1 : 256 dilution, was used to study the characteristics of varying inhibitory factors. The materials tested, and the findings are listed below:—

(*a*) Egg-albumin inhibited hæmolysis in dilutions up to 1 : 8.

(*b*) Cholesterol was titrated against the stock, hæmolytic, seminal plasma and showed no inhibitory activity. When it was diluted in a range 1 : 4 upwards in physiological saline solution, it induced lysis of red blood cells in the range of dilutions upwards to the limit of the test. The control tubes showed no lysis of the cells. In the presence of blood serum, 1 : 4 and diluted in the same range, the lytic action of the cholesterol was inhibited. Further, blood serum in dilutions up to 1 : 512 was shown to inhibit the lytic activity of cholesterol diluted 1 : 4,000 in physiological saline.

(*c*) It was found that Bovine Fraction V, a standard protein fraction of blood serum, showed only a trace of inhibitory activity in a dilution of 1 : 4 when tested against a 1 : 64 dilution of the stock hæmolytic seminal plasma. Bovine Fraction V, diluted in the presence of bovine red blood cells, caused lysis at dilutions greater than 1 : 2,048. Bovine Fraction V, at a constant dilution of 1 : 4, was tested against cholesterol in doubling dilutions, upwards from 1 : 4, through twenty-four tubes. Lysis of the red blood cells did not occur, in spite of the potential hæmolytic activity of cholesterol. Thus, it appeared that Bovine Fraction V is able to inhibit the hæmolytic property of cholesterol but not of hæmolytic seminal plasma.

(*d*) Further tests were carried out to determine whether the genital mucus of heifers contained a factor which would inhibit the hæmolytic activity of seminal plasma. Mucus samples were collected from eleven heifers of varying

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degrees of genital health and it was found that none of the samples inhibited the hæmolysin present in seminal plasma under conditions in which blood serum did so.

A series of tests were carried out to demonstrate the possible presence of hæmolytic factors in the genital mucus of heifers. These tests failed to reveal the presence of hæmolytic factors.

Attempts were then made to determine whether the genital mucus collected from heifers known to be suffering inflammatory changes of the genital tract might be hæmolytic. It was again found that no hæmolysis occurred.

Discussion

The above findings lead to a number of interesting possibilities and to a number of questions which remain to be answered in the future. It has appeared that semen samples from healthy, fertile bulls in full use are non-hæmolytic immediately after collection. There is a tendency, however, for the first ejaculate to be hæmolytic if the bull is not used to the full capacity of his mating ability, subsequent ejaculates on the same day being free from hæmolytic factor. Following the collection of such non-hæmolytic samples of semen, hæmolytic activity increases as the viability on storage decreases. The rate of development of hæmolysin is relative to the temperature of the material during storage. Thus, at room temperature non-hæmolytic semen becomes hæmolytic in 24 to 48 hours, whereas at —20° C. its development is inhibited indefinitely. When we have tested the ejaculates from bulls with a history of a poor conception rate, the semen being of poor viability on storage, they have been found to be hæmolytic immediately after collection. We have recorded an instance in which the first three ejaculates from a young, previously unused bull were hæmolytic. This finding is in accordance with the observation that the ejaculates from fertile bulls tend to be hæmolytic during phases of lack of full use. The development of hæmolytic factor in the seminal plasma would then appear to be the result of a natural physiological process which may proceed either *in vivo* or *in vitro* when the temperature environment is sufficiently high for katabolism to proceed.

The laboratory tests have shown that the presence of spermatozoa is unnecessary for the production of a hæmolysin in seminal plasma. In bulls suffering subacute inflammatory changes of the seminal vesicles there would seem to be a tendency for katabolism of the vesicular secretion to proceed at a faster rate, due to the presence of bacteria. However, the resulting seminal plasma hæmolysin is distinct from bacterial hæmolysin, although both may be present in grossly contaminated samples of semen. It may be that the process of oxidation of the seminal plasma produces a hæmolytic factor. This may explain why the passage of non-hæmolytic seminal plasma through an asbestos pad induces the production of a hæmolysin by allowing a large surface area to be exposed to the atmosphere. Further, it has long been recognised that semen can be stored most successfully when in tubes which permit only a relatively small surface area to be exposed. The application of liquid paraffin to this small surface gives added protection.

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Summary

It has been noted that a hæmolytic substance may be present in bovine semen. This observation may have some future value either in the evaluation of the genital health of bulls or in the assessment of individual samples of semen intended for storage at artificial insemination centres.

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STUDIES ON HÆMORRHAGIC SEPTICÆMIA OF CATTLE

VI.—Experiments with Oil-adjuvant Vaccine

By R. V. S. BAIN, B.V.Sc., M.Sc.

Department of Veterinary Pathology and Bacteriology, University of Sydney, Australia

Introduction

Some experiments of short duration with oil-adjuvant vaccine have been reported by Bain (1954 *a* and *b*). This paper describes some tests conducted over longer periods in Thailand and Australia. Results of a related but independent trial in India have been published by Vancheswara Iyer, Gopalakrishnan and Ramani (1955).

Methods

These were generally as described in previous papers in this series. Naturally immune animals were excluded as far as possible from the experiments by the application of the agglutination test. This is important in countries where hæmorrhagic septicæmia is enzootic. In Australia, the disease is not known to exist. The adjuvant vaccines were prepared as described by Bain and Jones (1955). In brief, they consisted of formalised bacterial suspensions grown either on agar or in broth, and standardised to a density between 6 and 7 on Brown's opacity scale. The suspensions were emulsified in Shell "Ondina 17" liquid paraffin and lanoline, and were injected intramuscularly in doses of 2 to 3 ml., which is roughly equivalent in bacterial content to 5 to 10 ml. of conventional broth bacterins. It has been the aim in these experiments to keep the dosage to the smallest level. A vaccine would only be considered worthwhile if it could be made easily and economically in large amounts.

Animals were challenged by inoculating them subcutaneously in the neck with the appropriate dilution of a six-hour broth culture of *Pasteurella multocida* type I, "Insein" strain.

Experiment Using a 2 ml. Dose

In the short-term experiments previously described, very good results had been obtained with a 2 ml. dose of oil adjuvant vaccine. The average time between inoculation and challenge was six weeks, and the challenges were from

25,000 to 50,000 L.D. subcutaneously. At Muaklek in Thailand, 84 adult buffaloes were inoculated with 2 ml. of agar-grown vaccine from a batch which had protected 15 out of 16 buffaloes challenged at the fifth week. The intention was to withdraw six animals for challenge at intervals of two months, starting

TABLE I

Response to challenge of buffaloes receiving 2 ml. of adjuvant vaccine.

Group	Time of Challenge	Challenge Dose	Survivors	
			Experimental	Controls
A	13th week	25,000 L.D.	3/6	2/2+
B	13th week	25,000 L.D.	2/6	2/2+
C	17th week	5,000 L.D.	1/6	0/2
D	17th week	500 L.D.	1/6	0/2
E	18th week	50 L.D.	3/6	0/2

Numerator = survivors; denominator = number in group.
*These are the animals which acquired immunity naturally during the experiment. For discussion, see Paper I in this series.

three months after vaccination. Control unvaccinated animals were kept in the herd and two were to be withdrawn for challenge with each group. The results are summarised briefly in Table I.

The results in groups A and B were not as good as was expected and the remaining animals were subjected to the agglutination test 15 weeks after vaccination. This showed that few of the animals were likely to be immune and several groups were then challenged forthwith with varying doses of virulent bacteria. The losses were heavy, and it was apparent that the immunity which they had probably enjoyed at the end of the first month had almost disappeared by the fourth month after vaccination. There was some fibrosis at the site of inoculation in the gluteal muscles in the animals which died, but unabsorbed vaccine could no longer be seen.

Experiments With 3 ml. Doses

It had been found in some preliminary tests that, as expected, the larger the dose of oil adjuvant vaccine, the better the antibody response. When the 2 ml. dose failed to fulfil the promise of the short-term experiments, larger doses were tried, but always with due regard to practicability. The author's assumption has been that the final method of production would be based on aerated broth cultures which attain a density of 7 on Brown's opacity scale without any need for concentration. Hence, we have avoided reducing the total volume of the dose by mechanical concentration of the bacterial suspension, preferring to work with the densest suspension which can be produced by direct cultural methods. It is not feasible to produce the suspension on a very large scale from solid media. Another possibility of improving the response to a small dose of vaccine is the inclusion in the continuous oily phase of some killed *Mycobacterium butyricum*. This was tried in these experiments.

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Most of the animals received a dose of 3 ml.; one small group only receiving 5 ml. Three small trials were made, two in Thailand involving 10 cattle and 10 buffaloes with control animals, and one in Sydney using 39 cattle. The animals in Thailand were challenged after six months with approximately 1,000 L.D., but those in Australia could not be challenged and, for them, indirect tests for immunity had to be worked out, as reported in No. 5 of this series. Before allocation to the experimental groups, all the animals were tested carefully

TABLE II

Response to challenge of buffaloes and cattle receiving 3 ml. of oil-adjuvant vaccine.

Group	Time of Challenge	Challenge Dose	Survivors	
			Experimental	Controls
Cattle	25th week	1,000 L.D.	10/10	0/2
Buffaloes	26th week	1,000 L.D.	10/10	0/2

for naturally acquired immunity, and any which reacted significantly to the agglutination test were eliminated. For the tests in Thailand, a dose of 3 ml. oil adjuvant vaccine was used with 0.025 per cent of *Myco. butyricum* added to the oil before blending. In Sydney, several vaccines were tried, but all were standardised to the bacterial content of 3 ml. of oil adjuvant vaccine.

The results obtained from the Siamese buffaloes and cattle are shown in Table II. The vaccine had been stored for six months in a domestic refrigerator before being used and both groups were inoculated with different batches of agar-grown vaccine containing *Myco. butyricum.*

All the immune cattle showed mouse protective and agar-agglutinating antibodies at the time of challenge. The buffaloes were tested for mouse protective antibodies only, and all the immune animals proved positive.

Whilst these tests were being made in Thailand, experiments were started at the University of Sydney Farm to find ways of improving the vaccine whilst keeping the dose to practicable limits. The animals available were Jersey-cross heifers and many were in calf. Immunity could be assessed by indirect methods only, and at present the mouse-protection test is considered the most reliable for this purpose (Bain, 1955). The results are summarised in Table III.

In the mouse protective test, 0.5 ml. of serum from each bovine animal is injected into each of four mice. It has been the experience, so far, that survival in the mice indicates an immunity up to a challenge with 1,000 L.D. in the test animal.

Of the heifers inoculated with oil-adjuvant vaccine, 29 out of 30 were considered immune at the end of the sixth month. Some of the animals were then sold, others calved and went into the production herd, and routine testing was suspended. However, 45 weeks after vaccination, 24 of the 29 immunes were retested and 23 continued to show mouse protective antibody.

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The animals in Group 6 (Table III) received 2 ml. of plain bacterin, which was approximately 20 per cent more than the dose received by Groups 1 and 3, though these had theirs in adjuvant. Six weeks after inoculation, only two of eight animals could be judged immune, and at the sixth month only one, which, however, continued to show mouse protective antibody at the eleventh month.

TABLE III

Response of Australian cattle to various oil-adjuvant vaccines, tested 25 weeks after inoculation.

Group	Vaccine	Dose	Mouse protective antibody	
1	Broth-grown + Myco. butyricum	3 ml.	8/8	(50)
2	Broth-grown + Myco. butyricum	5 ml.	4/4	(94)
3	Broth-grown alone	3 ml.	7/8	(44)
4	Agar-grown alone	3 ml.	5/5	(80)
5	Agar-grown + Myco. butyricum	3 ml.	5/5	(60).
6	Plain bacterin (same suspension as in 1, 2 and 3 without adjuvant)	2 ml.	1/8	(6)

Numerator = Number of cattle showing mouse protective antibody.

Denominator = Number of cattle in group.

In brackets = Percentage of mice surviving in each group (4 mice to each heifer per test and test done in duplicate).

Discussion

It is not possible to compare closely the results with 2 ml. vaccine and 3 ml., as different batches of vaccine were employed although all were made from the same strain of pasteurella. The most probable points of difference between batches of oil-adjuvant vaccine would be the quality and stability of the emulsion. Although one can be sure that the emulsion is of the desired type and that the droplets of disperse phase are a reasonable size, there may be less obvious properties which could affect, say, the rate of absorption. The batch of vaccine used as a 2 ml. dose was capable of initiating an immune response, and had immunised 15 out of 16 buffaloes successfully. In India, 2 ml. of similar vaccines prepared from other strains of type I *P. multocida* have conferred immunity on buffalo calves and hill cattle for up to eight months (Vancheswara Iyer *et al.*, 1955; Dhanda and Das, personal communication). These investigators were working with animals which had not been tested for naturally acquired immunity, though their experiences suggested that this was not a great problem in their areas. The improved response to the 3 ml. dose in our experiments could be caused by the larger dose or by some unknown factor in the vaccines. The improvement in duration of immunity occurred with four different batches of vaccine, grown

either on agar or broth, with or without added mycobacterium, so it seems that the increase in dose is probably important. This is in accord with the general behaviour of killed vaccines. The effect of a larger dose of *P. multocida* bacterin on improvement of immunity was brought out by Rau and Govil (1950); a large batch of oil-adjuvant vaccine was distributed in S.E. Asia for use in a field trial. In one centre, a small laboratory-type experiment was done on buffaloes using a 2 ml. dose, and three out of six survived two months later. Another centre used the recommended 3 ml. dose, and 10 out of 10 survived two months later, the level of challenge being approximately equal.

From the results of the Sydney experiment (Table III), it is impossible to deduce whether the addition of *Myco. butyricum* has been of any value. In experiments on mice, no benefit has been observed by incorporating *Myco. butyricum* in the oil phase of oil-adjuvant vaccine.

Effective vaccines may be prepared from bacteria grown in aerated broth. Agar-grown bacteria are better than broth-grown for inducing the production of complement-fixing antibodies (see Paper V of this series). It is not convenient to use solid media for large-scale production, and any deficiencies inherent in broth-grown bacteria would be offset by the greater convenience of preparation by growing in an aerated liquid medium.

In all these experiments, there have been no adverse reactions to oil-adjuvant vaccine. Doses up to 10 ml. intramuscularly have been tolerated without local swelling or lameness. It would be quite feasible to give doses larger than 3 ml. to cattle, and this could be done to valuable animals with prospects of obtaining an enhanced immune response. In the light of the results of the preliminary experiments described here, a 3 ml. dose of oil-adjuvant vaccine has been selected for use in a field trial in S.E. Asia in the 1955 rainy season. At the time of writing this paper, over 30,000 cattle and buffaloes have been vaccinated in Thailand, Burma and India, and the effect will be followed for the next year or two.

Summary

A 3 ml. dose of oil-adjuvant hæmorrhagic-septicæmia vaccine as previously described, has immunized cattle and buffaloes as judged by a direct challenge 6 months later. The same dosage induced the formation of mouse protective antibodies which could be found in the blood of cattle 11 months after inoculation.

Acknowledgments

The author is grateful to Mr. H. J. Geddes of the Sydney University Husbandry Farm for the supply of experimental cattle. Special thanks are due to Dr. Prayoon Boonmongkol and Dr. Veset Kongrod of Pakchong, Thailand, for making some of the inoculations and tests described in this paper.

REFERENCES

Bain, R. V. S. (1954a): *Nature, 173,* 584.
Bain, R. V. S. (1954b): *Bull. Off. int. Epiz., 42,* 256.
Bain, R. V. S., and Jones, R. F. (1955): *Brit. vet. J., 111,* 30.
Bain, R. V. S. (1955): *Brit. vet. J.* In press.
Das, M. S. (1955). Personal communication.
Dhanda, M. R. (1955). Personal communication.
Govinda Rau, K., and Govil, J. L. (1950): *Ind. vet. J., 27,* 105.
Vancheswara Iyer, S., Gopalakrishnan, K. F., and Ramani, K. (1955): *Ind. vet. J., 31,* 379.

XUM

STUDIES ON CONTAGIOUS BOVINE PLEUROPNEUMONIA

With Special Reference to the Complement-Fixation Test

By R. MOYLAN GAMBLES, M.A., B.Sc., M.R.C.V.S.
Senior Veterinary Research Officer, Vom, Nigeria

PART III

(vi) *Serological reactions in cattle supposed to be immune.* Six animals which had previously been vaccinated or had survived inoculation with virulent cultures (in most cases with a marked serological reaction), and which had subsequently been exposed to natural infection without developing anything more than a transient partial reaction, were each given 1 c.c. of freshly reconstituted dried pleural exudate subcutaneously, as were two susceptible controls. The latter both developed typical reactions, titres of 1/320 and large swellings necessitating slaughter. The vaccinated animals developed only negligible reactions (two partial and the rest traces or nothing).

Subsequent observations have confirmed that fully immune animals do not usually give serological reactions to subcutaneous inoculation with dried exudates. A difficulty arises at this point as to what may be considered a fully immune animal, as breakdowns to vaccination are known to occur and the immunity of a vaccinated animal cannot be taken for granted. Consequently, only animals which have survived inoculation with virulent material have been accepted as immune. Six such animals have been challenged with dried exudate on various occasions and have shown no serological reactions. A seventh animal (No. 1625, to be discussed later), which had survived a virulent inoculation, was found on further challenge to be still partly susceptible in that it not only reacted serologically but also developed a local reaction at the site of inoculation.

Two similarly immune animals, survivors of a previous challenge with dried exudate, were inoculated subcutaneously with tumour lymph from a reacting control animal in one of the previous experiments. These reacted in the same way as the animals of insusceptible species mentioned above, not only developing titres (in this case 1/40 and 1/80) but developing them by the seventh day, when susceptible animals seldom show anything more than a trace. The higher reactor showed a moderate-sized soft œdematous swelling at the site a few days after inoculation. This was not particularly like the typical local reaction to pleuropneumonia exudates, and quickly subsided. This experiment was repeated four months later, using one of the same two animals (the other had meanwhile died from other causes), and one of those that had received dried exudate without reacting. The former reacted similarly to before, but with a lower titre (1/20). The latter failed to react at all, showing that a serological reaction to fresh exudate is not invariable.

(vii) *Titres observed in unheated serum.* Karmann and Witte (1926), some of the earlier workers on the serology of pleuropneumonia, recorded that they obtained a higher number of positive reactions when they tested unheated sera instead of those that had undergone the customary "inactivation" by heating

at 56° C. for half an hour. This finding was briefly quoted by Campbell and Turner (1936) without comment, and its significance seems to have been overlooked by all subsequent workers. The same observation was made independently in the course of the present investigations, and it was found that, in all but very high titre sera, the titre of an unheated serum was usually two or three tubes higher than that of the heated. This is, to say the least, unexpected. The unheated serum might well be expected to contain a certain amount of natural ox complement, and this might be fixed by the antigen-antibody mixture in the test, leaving some of the guinea-pig complement free to lyse the sensitised cells of the hæmolytic system. This would make a positive serum appear negative or at least of lower titre than if it had been first inactivated by heat. In practice, however, the opposite is seen. Low titre sera appear higher if tested without heating, and some —though by no means all—negative sera may appear positive. This is not due (except in a few cases) to heat-labile anticomplementary substances. Using a control tube (without antigen) with every serum dilution, it can quickly be seen to what extent the effect is due to anticomplementary substances—in which case the complement is destroyed in the control tube also—and to what extent it is due to specific fixation of complement by antigen and antibody.

This effect is not due to any of the antibody being destroyed by the heating. but to the destruction of some titre-enhancing factor which is present in normal ox serum. When a serum which when unheated is positive for specific pleuropneumonia antibody has had its titre reduced or destroyed by heating at 56° C. for half an hour, the addition of unheated serum from a normal negative animal (often even in quite small quantity) will restore the titre to its original level. This enhancing factor has been found in a single sample of rabbit serum examined, to the same extent as ox serum. It has also been found to a slightly lesser extent in sheep (two examined), goats (one out of two examined), and horses (one out of two). Further investigation may show this titre-enhancing factor to be of widespread occurrence in various species of animal, and by no means confined to pleuropneumonia antigen and antibody. Christian, Ervin and Young (1951) have recorded that a heat-labile constituent in the fresh serum of normal dogs enhances the activity of type A iso-hæmagglutinins, but not those of other types. They also noticed that a heat-labile constituent of normal sheep serum caused a similar though less well-marked enhancement of the agglutinative capacity of rabbit anti-sheep-cell serum. Their work was repeated by Stohlman (1953), who found that the enhancing factor for the iso-agglutination of canine type A cells was also present in fresh ox serum, but not in that of the human and the guinea-pig. He argued that this enhancing factor was a component of complement, because it was lost after the serum had been treated with sensitised dogs' cells.

These titres in unheated sera were first observed during the present work in serum of animals which had recently been positive to the C.F. or rapid-slide test, or both, but which had since become negative. Subsequently, whenever time permitted, all sera for the C.F. test were tested both heated and unheated. It was found that the titre of the unheated serum could be detected, as a rule, earlier during the course of a reaction than that of the heated, and usually it persisted

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longer. Positive reactions were occasionally observed, however, in the unheated serum of negative animals which had never been either vaccinated or in contact with the disease. A satisfactory explanation of this lack of specificity has not yet been found, but it may be related to the general property of unheated mammalian serum which Mackie and Finkelstein (1930) found would often fix complement with a wide range of bacteria. The test with unheated serum is therefore of little use as a normal diagnostic agent, but where eradication is attempted by frequent testing and removal of early reactors, tests with unheated serum would probably lead to earlier detection of cases, even if in a certain number of instances animals were isolated as suspects without subsequently becoming positive to the normal test using heated serum. A more important application is in the selection of susceptible animals for experimental purposes. Where the unheated serum is positive to the C.F. test, the animal may be considered as of doubtful susceptibility, and unsuitable for experiment.

B. Challenge experiments (using a technique based on the above observations).

Small-scale experiments using the agar embolus technique of Mettam and Ford (1939), the intravenous chloroform technique used in goats by Longley (1951), and an atomised spray technique based on Campbell (1938*a*) and Hobday (1945), were disappointing or uncertain, although observations were made of sufficient interest to be worth recording. In no case were lung lesions set up by the intravenous methods, and there were a number of local reactions at the site following leakage of virulent material through the damaged vein. In the embolus experiments (using dried exudates resuspended and then solidified with agar), two known immune animals were included. Both of these developed titres, unlike those given dried exudates subcutaneously in the experiments described above. A possible explanation of this is that the agar plugs, containing not only dried organisms but also the nutrients from the original exudates, acted as a culture medium, and that active multiplication had commenced before immune bodies from the host had diffused in.

The experiment with spraying was carried out in conjunction with Mr. A. L. C. Thorne. Four animals were raced round the yard and then, while they were still panting, two were sprayed, into the nostrils, with an 8th-generation culture of a virulent strain isolated by Mr. Thorne from a case in Adamawa, and two with reconstituted dried pleural exudate. A "Sparklet" aerosol sprayer was used. The left lung of each animal was then punctured with a stout 4-inch sterile hypodermic needle, to cause slight mechanical damage where the organism might have an opportunity to localise itself. The first of the Adamawa animals developed a titre with a peak of 1/80 on the 13th day, falling to a trace by the 32nd day, when it was slaughtered. No sign of any lesion past or present was noted at post-mortem. The second animal remained negative for five weeks, but developed a titre of 1/160 after six weeks and was slaughtered on the 59th day. There was a nodular lesion at the site of the needle puncture from which the organism was re-isolated, but it was not by any means a typical pleuropneumonia lesion. Neither animal showed any clinical symptoms. The two sprayed with dried exudate were stabled together apart from the others and for 12 weeks

showed no serological reaction. One was positive at the 13th week (1/80 rising in two days to 1/160) and was slaughtered a week later. It was found to be a typical case of acute pleuropneumonia, and one lung (*not* the one punctured by the needle) was completely solid. The other animal was subsequently infected, but it is not possible to say whether this resulted from the original spraying or from contact with the first case. The long incubation period, without even any serological reaction, is interesting but not unprecedented. Seddon (1953) quotes cases of the disease in which the serum was negative for at least 70 to 80 days after the last exposure to infection.

Doubtless with more experience of this method of challenge better results would have been obtained, but even in Australia (Campbell and Turner, 1953), it appears that a certain number of animals exposed to sprayed virulent culture develop no clinical signs, not even pyrexia, and show no post-mortem lesions. They merely develop high titres which are also seen in immune animals challenged by this method.

The present author, in common with workers in the Sudan and Kenya, has therefore continued with the much simpler subcutaneous method, but in Nigeria this has been modified in the light of the serological observations recorded above. Since after suitable forms of immunisation a condition can be obtained similar to that seen in naturally insusceptible species (viz., provided that *dried* virulent material is used as the challenge, *no* serological response follows), this absence of serological reaction has been taken as the criterion of solid immunity. Animals showing no local reaction but reacting serologically are considered to be partially immune, and will probably have at least some powers of resistance when exposed to natural infection under field conditions. By the orthodox procedure of subcutaneous challenge such animals would have been accepted as completely immune. The modified technique is thus much more severe than the previously accepted method, and some workers may consider it unnecessarily so. However, since the main purpose of challenge experiments is to compare the efficiency of vaccines, and so long as vaccines can be found which will stand up to such a severe challenge, this undue severity is hardly to be considered a disadvantage.

Some results will now be quoted of vaccine-testing experiments in which this method of challenge was used.

Fig. 3 shows the reaction to vaccination and to challenge in the first of these, the pilot experiment with KH3/J and JG referred to in Section *A* (iii) above. The reactions to JG are omitted, as this failed to immunise. In this figure, animals receiving a 2 c.c. dose and reacting well are seen to be solidly immune to challenge five months later, while those receiving only 1 c.c. show varying degrees of susceptibility, the more marked the reaction to vaccination the greater being the resistance to challenge. In later experiments, using more animals, results were not so clear-cut, owing to variable and anomalous reactions in some of them, but the general underlying pattern appears to be the same. One of the three animals given 1 c.c. of JG and that given 2 c.c., with no reaction or only a slight trace, were fully susceptible to challenge and died of the local reaction after three and six weeks respectively. Another of those given 1 c.c. of this strain with only a slight

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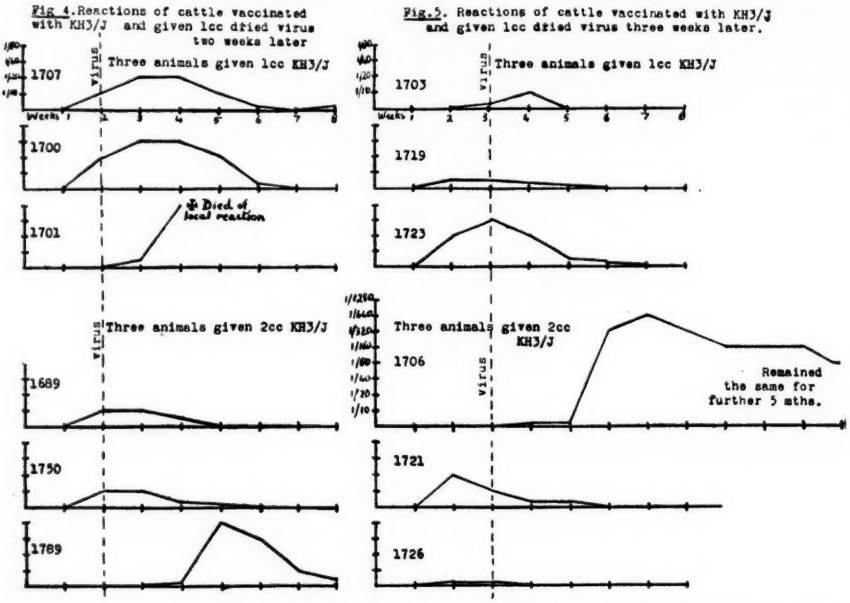
trace of reaction, No. 1625 (the third had died in the meanwhile), showed no reaction to subcutaneous challenge. It seems unlikely that this animal could have been solidly immunised by a vaccine which failed so completely to protect the two others even with twice the dose, so it may possibly have been immune previously. The reactions of two unvaccinated controls in this experiment are also shown in the figure. No. 1655 was believed to be fully susceptible and reacted typically. The second control, No. 1732, was included intentionally to see how



it would react. Its susceptibility was in doubt, because on one occasion it had shown a transient partial C.F. reaction, and had for three consecutive weeks given a positive reaction to Newing and Field's rapid-slide agglutination test. The unheated serum had not been tested at this time, but at the time of challenge it still showed a partial reaction, although the inactivated serum was negative, likewise the rapid-slide test. This animal gave no reaction to the challenge, and was taken as solidly immune. Since then, any animal which has shown a transient reaction at any time, or which has shown a distinct titre in its unheated serum has been excluded from vaccine experiments.

The reactions of the animals in the main experiment to which this was a pilot have already been summarised in Table II, and will not be given in detail here, except for those which were subsequently given virulent material (henceforward referred to loosely as "virus"), either as a challenge or in hopes of reinforcing the immunity. Eighteen animals, half given 1 c.c. and half 2 c.c. of KH3/J, were divided into three groups and given 1 c.c. of dried virus subcutaneously after two, three and four weeks respectively. Details of their serological reactions are given in Figs. 4 to 6. It will be noted that none of those given

2 c.c. of vaccine developed any local reaction to the subsequent injection of virus. One of them, however, No. 1706, given virus three weeks after the vaccine, developed a persistent high titre, 1/640 after four weeks, falling to 1/80 at the end of the ninth week, and fluctuating around this level for a further five months. During the next four months it gradually fell, and the animal was finally slaughtered. During the whole of this time it remained in good condition and at no time showed any local swelling or clinical symptoms of pleuropneumonia.

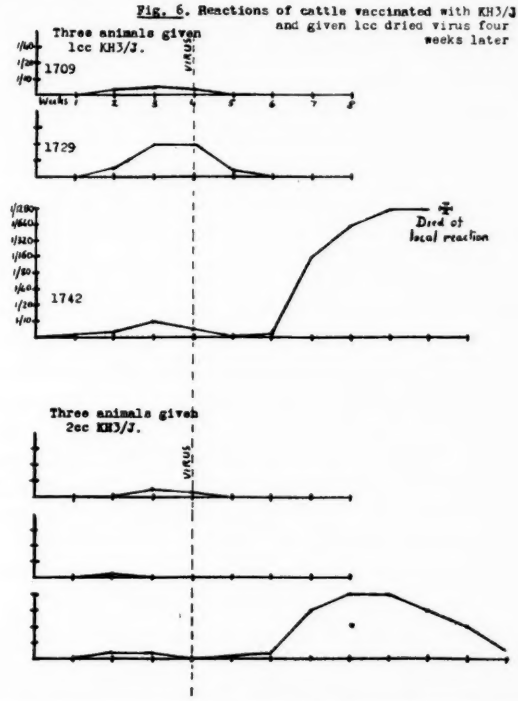


Fig. 6. Reactions of cattle vaccinated with KH3/J and given 1cc dried virus four weeks later

Owing to the persistent titre it was kept in isolation, suspected of still harbouring virulent organisms in the body and being capable of infecting other animals. At post-mortem examination, fairly extensive pleural adhesions were found in one lung, indicating the presence of pleuritic lesions at some time in the past. It is generally considered that lung lesions do not follow subcutaneous inoculation. A probable explanation is that the animal had already developed sufficient resistance to prevent the development of a local lesion, but not to prevent the organisms multiplying in the tissues and overflowing into the circulation to cause a transient bacteræmia. If some of the circulating organisms were held up in some slightly damaged portion of the lung (e.g., a parasitic or other lesion), in the absence of complete immunity these might have been able to establish themselves and set up lung lesions to a limited extent. Similar pleural adhesions were observed in No. 1316, the survivor of the six animals given fresh pleural exudate subcutane-

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ously (Table IV), which still had a titre of 1/80 when slaughtered 18 months after injection. In the latter case, however, the animal had not been partially immunised beforehand, so a more persistent lung lesion may have been set up, although it remained a subclinical case. The serological evidence suggests that a persistent focus of viable organisms was still present somewhere in the body, although such cultures of lungs or glands as were made were all negative.

It will be noted that of the six animals given virus two weeks after vaccination, four had already started to show antibodies, and in these there was no reaction to the virus beyond a slight increase in titre in two cases, and even this may have been part of the reaction to the vaccine rather than to the virus. The other two appeared to be still susceptible, that given 1 c.c. vaccine wholly so, and that given 2 c.c. partially so in that it gave a marked serological reaction to the virus. Of those given virus three weeks after the vaccine, No. 1706, which developed the persistent high titre referred to above (and probably also mild lung lesions), was the only one that had not already started to show at least a trace reaction to the vaccine. On the other hand, of those given virus four weeks after vaccination, one vaccinated with 1 c.c. died of a local reaction (although somewhat delayed and not wholly typical) and one given 2 c.c. showed a marked serological reaction; and both of these had shown at least a trace of antibody following the vaccine, with a titre of 1/10 in the case of the animal which succumbed. The suggestion that the commencing antibody production in the other animals was correlated with their resistance to the virus must therefore be taken with some reserve. The experiment shows that some animals (in this case four out of six) were already fully immune two weeks after vaccination, while others (two out of six) were still susceptible, wholly or in part, four weeks after. Two of those given 1 c.c. of vaccine followed by virus two weeks later were challenged again after seven months and were still solidly immune. The remaining animals had died meanwhile from other causes, and were not available for challenge.

Owing to the poor quality of the animals available for experiment and a considerable amount of intercurrent disease in the herd at that time, apart from the two just mentioned only eight other vaccinated animals lived long enough to be challenged seven to nine months later. The reactions of these animals to vaccination and challenge are shown in Table V.

Of three animals given a single dose of 1 c.c. KH3/J, two were partially susceptible after seven and nine months respectively. The latter had reacted well to the vaccine, so it may have had a reasonable degree of immunity at one time which was starting to wear off after nine months. The remaining animal, No. 1696, was probably solidly immune. It died from other causes during the fourth week after challenge, so the possibility remains that had it lived longer it might have shown delayed reaction. That this is unlikely, however, is shown by the fact that the unheated serum developed a titre of 1/40 after the second week and had fallen to 1/20 by the third week, suggesting that any tendency to react that there might have been was already diminishing by the time the animal died. Another animal vaccinated twice with 1 c.c. with an interval of

three weeks appeared to be solidly immune seven months later. Three animals given a single dose of 2 c.c. were solidly immune when two were challenged after seven months and the third after nine. However, an animal which had been given two 2 c.c. doses with an interval of three weeks showed a considerable

TABLE V.

Reactions to vaccination and subsequent challenge.

Animal number.	Dose of KH3/J.	Maximum titre.	Interval.	Challenge dose.	Maximum titre.	Local reaction.	Remarks.
1663	1cc	Partial	7 months	1cc dried virus.	1/10	Nil	Partly susceptible.
1696	1cc	1/20	7 months	"	Sl.trace	Nil	Died 4th week, other causes; probably solidly immune.
1725	1cc	1/20	9 months	"	1/10	Nil	Partly susceptible.
1685	1cc repeated after 3 weeks	1/20	7 months	"	Nil	Nil	Died 5th week, other causes, but solidly immune.
1672	2cc	Nil	7 months	"	Nil	Nil	Solidly immune.
1674	2cc	Trace	7 months	"	Nil	Nil	Solidly immune.
1679	2cc	1/20	9 months	"	Nil	Nil	Solidly immune.
1746	2cc repeated after 3 weeks	Sl.trace	7 months	"	1/320	Small swelling	Susceptible, but not fully so.
1922	Unvaccinated control	- - -	(7 months)	"	1/320	Fatal swelling	Fully susceptible.
1962	Unvaccinated control	- -	(9 months)	"	1/160	Fatal swelling	Fully susceptible.

degree of susceptibility at the end of seven months and developed not only a high titre after challenge, but even a small local swelling. This is hard to account for, and the possibility that the repeated dose of vaccine instead of immunising had in some way sensitised the animal, while unlikely, cannot be ruled out entirely. Apart from this, it appears that a 2 c.c. dose of this vaccine strain gives a solid and reliable immunity lasting for at least five to seven months. Exceptional cases, however, call for a considerable amount of further study before any firm conclusions can be drawn as to the certainty with which such vaccines will immunise.

(Part IV will appear in April issue)

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THE USE OF PHENYLBUTAZONE TO CONTROL PAIN IN VETERINARY PRACTICE

By P. J. DALTON, M.R.C.V.S.
Salisbury

PHENYLBUTAZONE (B.T.Z.) is one of a group of Pyrazole derivatives, 3,5-dioxo-1,2-diphenyl-4-n-butyl-pyrazolidine and because of its antihistaminic, antipyretic, analgesic and anti-inflammatory properties it has been used extensively by medical men in the treatment of painful musculo-skeletal disorders such as spondylitis, osteo-arthritis, muscular rheumatism, neuralgia, bursitis and inflammation of ligaments and tendons. In man, certain toxic side effects have been noticed, i.e., gastro-intestinal disturbances, water retention, maculo-papular rash and agranulocytosis, but they have not been reported in dogs. The administration of 200 mg./kg./day over a period of 11 weeks caused anorexia and a gradual loss of weight with low R.B.C. and hæmoglobin levels, but histopathological study of liver, spleen, kidney and bone marrow revealed no evidence of changes which could be attributed to the drug.

In man, three to four days are required to effect a stable blood level which disappears in an amount of 15 to 25 per cent per day so that four to six days are required for a complete elimination of the drug. In the dog metabolism occurs at a faster rate, and recurrence of symptoms occurs within 24 hours of cessation of administration. Lieberman reported his clinical findings in the treatment of about 30 dogs affected with posterior paralysis, arthritis, painful injuries and fractures to limbs and joints. He obtained highly satisfactory results.

Over the last 12 months phenylbutazone has been used in this practice for a variety of conditions in which pain was a prominent symptom.

Case 1. A five-year-old male cocker spaniel. The shoulder joint had been injured some three months previously. Very little weight was being taken on the affected limb and muscular atrophy had occurred. 200 mg. phenylbutazone were administered orally thrice daily and within 48 hours the dog was chasing rabbits. Within 48 hours of withholding the drug the animal was in pain again and walking on three legs. Improvement occurred when administration was repeated.

Case 2. A nine-year-old Labrador bitch, suffering from arthritis of the stifle joint. X-ray films showed "lipping" of the tibia. This gun-dog can work a full field day if four doses of 200 mg. of phenylbutazone are given on four occasions and at eight-hourly intervals on the day before she is due to work and if one dose is given on the working day.

Case 3. A two-year-old Corgi stud dog which had suffered a fracture of the posterior third of its ospenis which was not detected until some months later.

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Intermittent service had been permitted so that union had not occurred at the fracture site. The dog was becoming apprehensive of serving and exhibited manifest pain when it turned after tying.

Two doses of 200 mg. phenylbutazone followed by one of 100 mg. at eight-hourly intervals commencing 24 hours before service banished the pain. The drug appeared to interfere with surface sensation so that the dog was not able to appreciate the location of the vulva. It is hoped that by regulation of the dose this factor will be overcome.

Case 4. A ten-year-old ginger neutered male cat which had exhibited a sudden acutely painful arthritis condition of the elbow the day after it had killed a stoat, which, it is presumed, had bitten it through the joint. Parenteral penicillin did not influence the condition, but great relief followed the intra-capsular injection of penicillin and streptomycin in a mineral oil with aluminium stearate (Streptopen Glaxo) but this wore off in three weeks and a second injection did not have the same salutary effect. When 50 mg. phenylbutazone is given twice daily most of the pain and lameness is abolished and a maintenance dose of 50 mg. once daily permits the cat to use the leg with only a slight limp.

Case 5. A four-year-old male terrier which had been hit at close range by a load of shot from a .410 shotgun. About a dozen pellets were embedded around the top of the left hind leg and pelvic area. There was a discharging sinus one quarter of an inch dorsal to the tuber coaxæ. The dog carried the leg continuously and there was muscle atrophy. Phenylbutazone was given at the rate of 100 mg. thrice daily. It made little difference to the amount of use made of the limb, but the dog's general well-being was much improved so that for the first time in six months it once more goes rat-hunting.

Case 6. A six-year-old male Scottie suffering from a very painful postho-balanitis. The back was arched, the gait was stilted and there was intense pain when pressure was applied to the root of the penis. The dog was given 3 x 100 mg. of phenylbutazone and the symptoms were relieved completely within 24 hours.

Case 7. A ten-year-old Sealyham bitch in pain due to a mammary carcinoma. The growth had recurred after surgical removal and had been referred to us for X-ray irradiation. The very high dosage of X-rays employed produced an irradiation burn. There was considerable cicatrisation and this gave rise to an increase in the pain and a limitation of gait. Phenylbutazone in dosage of 400 mg. per day did not prove of benefit in this case.

Case 8. A ten-year-old Cairn which suffered extreme ear irritation due to "Polyps" which had not been removed when a bilateral "Zepp" type aural resection had been performed. Phenylbutazone 200 mg. twice daily had no effect on the condition.

Other patients suffering from arthritic lesions, luxation of intervertebral discs, and painful myositic conditions have responded well to the administration of phenylbutazone,

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In only one case, a nine-year-old female bulldog with a bilateral arthritis of the stifle has a seeming resistance to the drug being encountered. A course of phenylbutazone was given when the first leg became affected. The drug was discontinued after it had restored the dog's ability to exercise sufficiently in order to reduce its excess weight. When the second leg became affected, following an accident, the drug did not appear to afford any relief.

It is intended to use this drug in other conditions in which pain is the predominant symptom, such as arthritis in pigs. It may prove of great economic value in the case of stud boars which are affected with a painful arthritis.

In none of the cases treated have any untoward side effects been noted, and the practical dosage level is so much lower than that needed to produce toxic symptoms, that fear on this score may be unfounded.

Summary

1. Phenylbutazone has been proved of value in the treatment of painful conditions in dogs and cats, especially those which involve the musculo-skeletal system.

2. Dosage for the dog appears to be in the range of 100 to 400 mg. per day, in divided doses commencing with a rather higher initial loading dose.

3. No toxic effects have been observed.

4. The drug appears to exert a specific analgesic action on certain types of pain, but had little effect when the pain was associated with cutaneous structures.

I would like to express my appreciation of the very generous supply of phenylbutazone (Butazolidin) made available by Geigy Pharmaceutical Company, Ltd.

REFERENCE

Liberman, L. L. (1954) : *J. Amer. vet. med. Ass., 125*, 128. August.

REVIEWS

THE ROLE OF ALGÆ AND PLANKTON IN MEDICINE, by M. Schwimmer and D. Schwimmer (Grune and Stratton, Inc.) Pp. x + 85. New York and London. Price $3.75.

THE medical and veterinary professions are not always well-informed on the role which is played by algæ and plankton. This book may go a long way to remedy this state of affairs. Employing an economical style of writing and presenting a considerable number of facts in tabular form, the authors have packed a great deal of information into a small compass. This information has been culled from an impressive list of papers and books most of which are post-war publications and more than half of which are post-1949.

The extensive field covers such diverse subjects as the use of agar for bacterial culture, the use of microscopic algæ for atmospheric control in atomic submarines, and the utilisation of algæ in oxidation in sewage disposal. Only a relatively small number of the known genera of algæ are included in this account. It may be that further research will reveal many more genera of algæ having medical importance.

Of great interest to the veterinary surgeon are two sub-sections (pp. 48-62) in which animal and human intoxications are discussed. Included are a table summary of 38 incidents of animal intoxication and references to 22 papers relating to these cases. The discussion of human intoxication, with table summaries and 38 references, is also of value.

The authors appear to express slight over-optimism regarding the mass-culture of phytoplankton as the "food of the future" and the attendant practical difficulties seem to have been overlooked. In closing, an interesting suggestion is made on the possibility that a symbiotic relationship exists between algæ and the virus associated with acute poliomyelitis. This, while it may fire the imagination of physicians, may possibly discourage prospective algologists!

This book was written in order to interest members of the medical profession in algæ and plankton, but there is no doubt that it will appeal to a much larger public.

WE acknowledge the receipt of a new veterinary textbook. It is entitled "Veterinary Parasitology" and the author is Dr. Geoffrey Lapage. It is published by Oliver Boyd, Tweeddale Court, Edinburgh (£3 3s.). The book deals with the helminth, arthropodan and protozoan parasites found principally in British farm animals. It is of about one thousand pages and is illustrated extremely well. A detailed review will appear in a later issue of this journal but a preliminary examination of the text and illustrations gives indication that the book will be of great value to the veterinary profession.

XUM

NEWS

COMMUNICATION of the Commission for organisation of post-graduate courses at the Veterinary College of Vienna. With a view to the fact that the X Congress of the International Scientific Film Association, I.S.F.A., whose III section (Veterinary Medicine) will be dedicated to the projection of veterinary educational films from all member states, will be held at Vienna from September 23 to September 29, 1956, the international post-graduate course of the Veterinary College of Vienna will not take place in 1956.—Prof. Dr. J. Schreiber (in charge).

VISIT TO AMERICA

DR. W. R. WOOLDRIDGE, Scientific Director of the Animal Health Trust, and Dr. K. C. Sellers, Director of the Trust's Farm Livestock Research Station, left Southampton on the "Queen Mary" on March 22 on a visit to the United States. The cost of the journey is being met from funds from American sources and its purpose is to make contacts with scientists in universities and industrial research establishments and to study the work being done for the benefit of animals and birds in the United States and Canada.

A keen interest will also be taken in veterinary education, particularly as concerns veterinary public health. A number of lectures will be given and an effort made to interest prominent Americans and organisations in the work of the Animal Health Trust.

Mr. J. Arthur Rank, the Chairman of the Executive Committee of the Trust, has issued a challenge to the effect that he will give £1 for every £1 donated in America up to a total of £15,000.

Drs. Wooldridge and Sellers will be away for two months, and it is hoped that they will bring back much information useful for the prosecution of research at the Trust's various centres in this country.

APPOINTMENT

MR. P. G. HIGNETT, lecturer in Veterinary Obstetrics and Infertility at Glasgow University, has been appointed veterinary consultant to British Glues and Chemicals, Ltd.

CHANGE OF ADDRESS

MESSRS. DUNCAN FLOCKHART wish to advise the profession that their London office, as from April 2, will be at 4 Carlos Place, Mayfair, London, W.1. P.O. box No. 227. Telephone: Mayfair 2355. Telegraphic address: Dunhart-Audley, London.

Publishers' Notices

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